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THE EFFECT OF THE TIME FACTOR ON THE AMOUNT OF PRESSOR MATERIAL PRESENT IN KIDNEY AFTER UNILATERAL LIGATION OF RENAL PEDICLE AND AFTER UNILATERAL LIGATION OF URETER[1]

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Increased amounts of pressor substance have been demonstrated in the kidneys of animals after complete (1) and partial (2) occlusion of the renal artery and also after ligation of the ureter (3). However, the effect of the duration of such occlusion or ligation has not been studied. It is the purpose of the present investigation to determine the relationship of the duration of *a*, complete ligation of the structures of the renal hilus, and *b*, occlusion of the ureter to the amount of pressor material present in the respective kidneys.

METHODS. In one group (a) of white rats, aged 3 to 4 months, the pedicle of one kidney was ligated, and in another group (b) one ureter was occluded. The animals in each group were sacrificed at varying intervals after the respective procedures. Extracts of both kidneys of each animal were prepared immediately after death by grinding the kidneys with the aid of powdered glass and putting the macerated tissues in 2 cc. of 2 per cent NaCl solution per gram of tissue and placing in a refrigerator for about fourteen hours. Then the material was adjusted to a pH of 4.5 and centrifuged. The supernatant fluid was tested for its pressor content by injecting it into white rats (weight 200–300 grams), anesthetized by 0.05 gram pentobarbital sodium per kgm. B. W., intraperitoneally. Blood pressure of these test rats was taken from a cannula in the carotid artery attached to a mercury manometer via a three way stopcock. One milligram of heparin (1 mgm. = 110 units) in 0.1 cc. saline was injected before the observations were made and blood pressure readings were taken at

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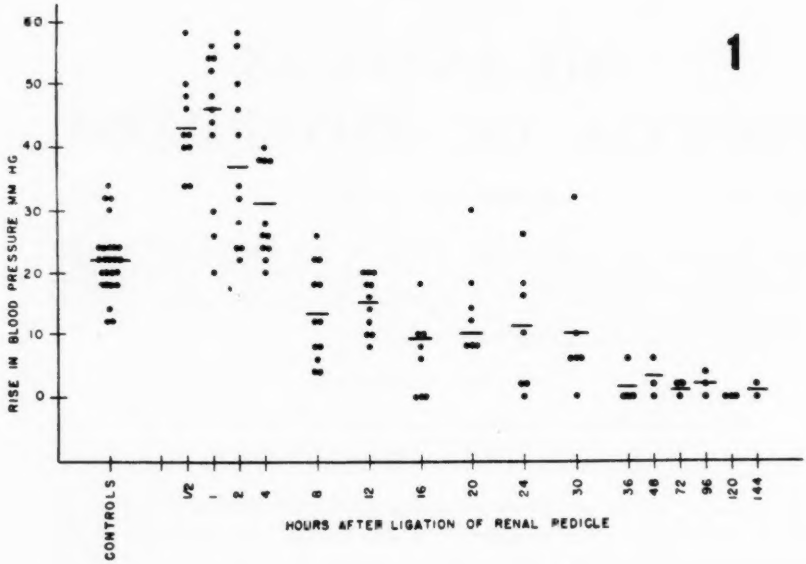
[2] Nemours Foundation Fellow.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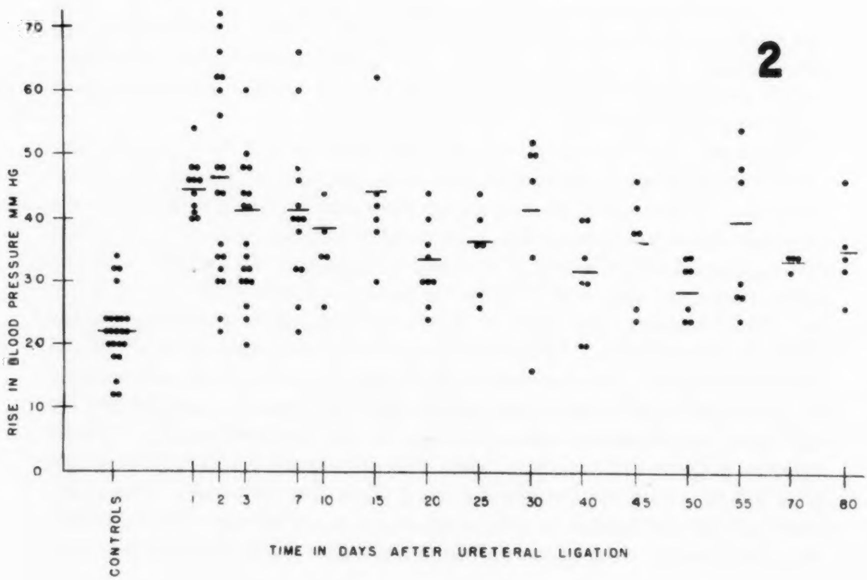
HOURS AFTER LIGATION OF RENAL PEDICLE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80
TIME IN DAYS AFTER URETERAL LIGATION

frequent intervals until a uniform pressure level was established. After stabilization of the blood pressure, 0.2 cc. of the extract to be tested was injected and blood pressure was taken every fifteen seconds for the first minute, every thirty seconds for the second minute and every sixty seconds for three additional minutes. The maximum increase in blood pressure above the basal level usually occurred in $1\frac{1}{2}$ to 2 minutes after injection and this figure was taken as the measure of pressor response.

Results and comment. a. *Unilateral ligation of renal pedicle* (fig. 1). The average rise in blood pressure produced by extracts of normal kidney was 22 mg. Hg. The average rise in blood pressure produced by extracts of kidneys removed $\frac{1}{2}$ and 1 hour after ligation of the renal pedicle produced an average blood pressure rise of 43 and 46 mm. Hg, respectively. This increase in pressor substance was presumably due to the fact that within the first hour, ligation of the renal pedicle caused ischemia of the kidney.

Extracts of kidneys removed more than 1 hour after ligation of the pedicle produced progressively less pressor response until extracts obtained more than 4 hours after ligation caused less average blood pressure elevation than did extracts of normal kidneys. A probable explanation of this observation would seem to be that enzymes after the first hour may inactivate the pressor substance.

b. *Occlusion of the ureter* (fig. 2). The normal controls used were the same as above. The greatest average blood pressure response in this group was 45 and 46 mg. Hg from extracts of kidneys removed 24 to 48 hours respectively after the ligation of the ureter. Ischemia of the kidney has been shown (4) to result shortly after ureteral ligation and presumptively this initial increase in pressor substance is due to ischemia of the kidney secondary to ureteral occlusion.

Extracts of kidneys with ureters ligated more than two days elicited progressively less blood pressure response. However, even 80 days after the ureter was tied, the kidneys contained amounts of pressor material greater than the average amount in normal kidneys. These kidneys were typically hydronephrotic and mere shells of tissue. Histologically, there was marked pressure atrophy and thinning of the cortex with round cell infiltration. The arteries appeared normal. Since there was increased pressor substance in the presence of atrophy of functional renal units, one might postulate that pressor substance was elaborated by some type of cell not involved in renal secretion. Such a postulation would be in accord with the work of Goormaghtigh and Grimson (5), who suggest possible endocrine cells in the kidney which secrete pressor material.

CONCLUSIONS

Kidneys removed within four hours after renal pedicle ligation contain greater than normal amounts of pressor substance. After this time the

amount of pressor substance decreases below that recorded for the control kidneys.

Amounts of pressor material in kidneys after ureteral ligation were greatest in the first two days after the procedure but remained in excess of normal in kidneys whose ureters were occluded for as long as eighty days.

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THE RÔLE OF THE HYPOTHALAMUS AND PREOPTIC REGION IN THE REGULATION OF HEART RATE[1]

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Since the discovery of the hypothalamus as a visceral center, numerous sympathetic functions have been ascribed to it (see review by Ranson and Magoun, 1939). But opinions differ as to whether cardiac acceleration also results from its stimulation. Jaegher and Bogaert reported in 1935 that the heart beats faster on stimulation of the hypothalamus; but no details were included. Kabat, Magoun and Ranson (1935) in their work on the vasomotor responses, could not obtain a uniform result on the heart, and they found only slight cardiac acceleration in 60 per cent of the cases in which stimulation brought about other sympathetic changes. But since the heart rate is definitely increased during emotional excitement and in the "sham rage" of decorticate animals (Cannon and Britton, 1925) and such spontaneous "rage" is known to originate from the hypothalamus (Bard, 1928), one would incline to believe that the hypothalamus takes an important part in cardiac regulation.

In our recent experiments with chloralose anesthesia, we have been confronted with numerous pressor reactions over 100 mm. Hg following the usual hypothalamic stimulation. Such high pressures were rarely obtained in our earlier experiments with sodium pentobarbital anesthesia. Since the previous work on cardiac rate was done on cats with the latter anesthesia it seemed worthwhile to repeat the experiments using chloralose as the anesthetic. The present report also includes a similar study on the preoptic region.

Methods. Sixty milligrams of chloralose per kilo of body weight was administered intravenously into each of the 24 normal cats. The preoptic region and hypothalamus were stimulated in the usual manner (Kabat et al., 1935). A bipolar nichrome wire electrode, completely enameled except at the tips, was introduced into the desired region with the aid of the Horsley-Clarke stereotaxic instrument. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 v) in the primary circuit with secondary coil at 9 to 9.5 cm.

[1] Aided by a grant from the Rockefeller Foundation.

The blood pressure was recorded from the right common carotid artery on a slowly moving kymograph. The speed of the drum was regulated so that the pulse could be easily recognized. The stimulus was given for a period of 30 seconds and the pulse was counted in intervals of 10 seconds, both before, during and after the stimulation. Various procedures, such as vagotomy, upper thoracic sympathetic ganglionectomy, adrenalectomy or tying of the abdominal vessels at the level of the diaphragm were carried out in some of the experiments and stimulation of the same region was then repeated.

The location of the reactive points was verified microscopically from a study of the serial sections stained by the Weil method.

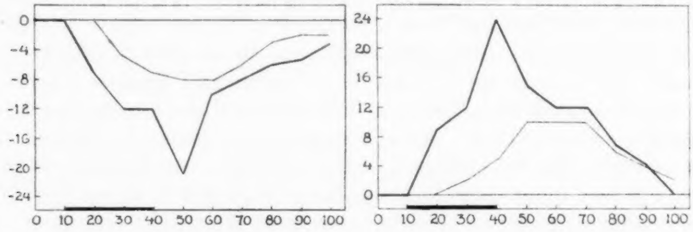


Fig. 1. Left. The effect of bilateral vagotomy on the cardiac slowing caused by stimulation of the preoptic region. Heavy lines, before vagotomy; light lines, after vagotomy. In this and other figures, ordinates denote the average per cent change of the heart rate per 10-second period; abscissae, time in seconds. The stimulus is marked at the bottom of these figures.

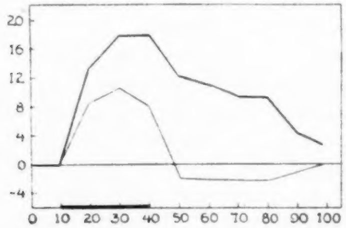
Fig. 2. Right. The effect of upper thoracic sympathetic ganglionectomy (from the stellate to T_5 ganglion) on the cardiac acceleration caused by hypothalamic stimulation. Vagi cut. Heavy lines, before ganglionectomy; light lines, after ganglionectomy.

Results. Slowing of the heart with depressor reaction was observed in 6 cats when the preoptic region was stimulated. The lowering of the blood pressure during the 30 seconds of stimulation varied from 18 to 30 mm. Hg; the number of heart beats decreased 6 to 19 per cent. The maximal drop during the stimulus usually occurred in the third 10-second period (fig. 1). On withdrawal of the stimulus, the heart slowed down further with slight additional dip of the blood pressure level. In the vagotomized animals the depressor response was not appreciably altered but there was less slowing of the pulse. Nevertheless, the slowing was still considerable after vagotomy (fig. 1). The effect for a 30-second stimulus would last for 2 or more minutes.

Stimulation of the hypothalamus, on the other hand, uniformly yielded among other sympathetic discharges an average increase of heart rate of 5 to 25 per cent over the control. The maximal acceleration usually

appeared during the third 10-second period of the 30-second stimulus and in several instances reached as high as 35 per cent over the control. If the vagi were intact, there was frequently a sudden slowing of the heart rate below that of the control period on withdrawal of the stimulus. However, such findings were not universal. The acceleration would in general last for 2 minutes or more, particularly in vagotomized animals.

In 3 animals the hypothalamic stimulation was repeated after excision of the upper thoracic sympathetic chain from the stellate down to the fifth or sixth thoracic ganglion. There appeared no cardiac acceleration during the first 10-second stimulation and also very slight during the second 10-second period. The post-stimulus quickening of the heart was, however, almost as good as that before the extirpation (fig. 2). In 3 other cats both adrenal glands were tied so as to exclude them from the general



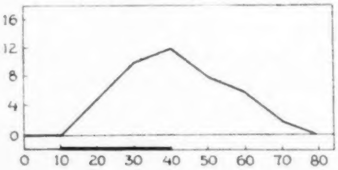


Fig. 3. Left. The effect of adrenalectomy on the cardiac acceleration caused by hypothalamic stimulation. Heavy lines, before adrenalectomy; light lines, after adrenalectomy.

Fig. 4. Right. Cardiac acceleration caused by hypothalamic stimulation after ligation of the abdominal vessels at the level of the diaphragm. No change of blood pressure was observed following the stimulation.

circulation. The cardiac acceleration lasted for a much shorter time after than before the adrenalectomy (fig. 3).

Since in all the experiments there was always a simultaneous occurrence of elevation of blood pressure and cardiac acceleration, a few experiments were performed on animals with the abdominal vessels, both the aorta and vena cava, tied at the level of the diaphragm (just above the right adrenal vein). In two of these animals, we were able to demonstrate cardiac acceleration on hypothalamic stimulation without accompanying arterial hypertension (fig. 4).

Comments. The persistence of the depressor response to preoptic stimulation after vagotomy indicates that the major effect is not manifested through the vagi. Since the response in the form of bradycardia is less marked in vagotomized animals, it is fair to assume that the slowing of heart is partly vagal especially that part of the slowing that occurs after withdrawal of the stimulus. Our evidence suggests the possibility that

the preoptic region exerts a dual effect, stimulation resulting in excitation of the vagus and inhibition of the sympathetic.

Stimulation of the hypothalamus caused a sustained acceleration of the heart, both through the cardiac sympathetics and through the splanchnics and the adrenal glands. The former is largely responsible for the earlier effect, the latter for the late effect. No attempt has been made to correlate the degree of cardiac acceleration with different hypothalamic nuclei at various levels of the diencephalon. But, in general, there is an apparent correlation between the responses of the heart and that of the peripheral vascular system. Our evidence suggests that in normal animals maximal cardiac acceleration is only obtained in cases where there is a marked pressor reaction. On the other hand, we have shown that the former is not necessarily secondary to the increased arterial tension, as it can be demonstrated differentially in animals in which the rise in blood pressure has been prevented by ligation of the abdominal vessels.

In view of the low-frequency reversal of vasomotor, pupillary and respiratory responses reported by Hare and Geohegan (1939), it was of interest to see if a slowing of heart rate could be obtained on low-frequency stimulation of the hypothalamus. In 2 cats thus stimulated[2] (unpublished data), a marked depressor response of 40 mm. Hg was obtained at the frequency of 5 per second, where a pressor response was obtained previously and subsequently with a high-frequency stimulus. In each instance a slight increase of heart rate was obtained. Vagotomy did not influence the response.

SUMMARY

An average increase of heart rate of 5 to 25 per cent was demonstrated following a 30-second stimulation of the hypothalamus. This tachycardia is largely effected through the cardio-sympathetic nerves and adrenin, and can be obtained differentially without the accompanying hypertension in animals with the abdominal vessels tied at the level of the diaphragm.

Stimulation of the preoptic region resulted in an average slowing of the heart of 6 to 19 per cent. The effect is partly vagal.

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[2] Stimuli obtained from a thyratron regulated condenser-discharge.

THE EFFECT OF EPINEPHRINE ON THE POTASSIUM BALANCE OF THE HIND LIMBS OF THE FROG[1]

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Since 1933 when Bachromejew discovered that the injection of epinephrine caused a rapid rise in the [K] of the blood of the cat, the researches of various workers have given us a well-defined picture of this phenomenal effect. D'Silva (7, 8) found in cats that the maximum [K] is attained in one minute after the injection, that this rapidly diminishes to the original level within about four minutes and may be followed by a considerable fall below the initial level within a further five minutes. A similar marked temporary rise in [K] followed by the fall to below the initial level was found in rabbits (18), in dogs (17) and in the human subject (1, 5, 6, 15). The exact time relations of the potassium response were found to vary with the species studied. We have concluded, on the basis of a series of experiments in which epinephrine was injected into sheep and goats, that these two species show the typical potassium response.

The increase in plasma potassium provoked by epinephrine is specific for that element since plasma sodium, calcium and ammonia remain essentially unchanged. The effect is seen with or without anesthesia, but in the latter case the effect is much greater.

Both D'Silva (8) on cats and Marenzi and Gerschman (17) on dogs gave convincing proof that the potassium in the response to epinephrine comes from the liver. In these studies the other organs of the body as well as the red blood cells were eliminated as producing the potassium.

The decrease in [K] following the rise is evidently due to the uptake of potassium by some organ or tissue. Marenzi and Gerschman (17) found that the muscles were active in this respect since the arteriovenous difference between the femoral artery and the femoral vein blood was found to be about +20 per cent one minute after the injection of epinephrine. A similar value (+20.67 per cent) was found by us in one out of two experi-

[1] This report is from a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Minnesota.

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ments in which the [K] of blood taken directly from the left ventricle of the unanesthetized dog by cardiac puncture was compared with that taken from the saphenous vein one minute after epinephrine. In a similar experiment on a dog under nembutal with the gastrocnemius muscle isolated the arteriovenous difference between left ventricle and femoral vein blood was +22.50 per cent at two minutes after epinephrine. In this latter case the possible uptake by bone or skin has been eliminated. In this connection, Houssay and Marenzi (13) found that the liver as well as the muscle gained potassium following the intravenous injection of KCl into dogs.

From the theoretical standpoint the most interesting phase of the potassium response to epinephrine is the after fall to below the initial concentrations. The initial rise seems to be the general result of a shocking agent, and our appreciation of that phase of potassium function is limited. But the after fall is paralleled by similar potassium changes occurring in connection with carbohydrate metabolism. Several investigators have reported the decrease in $[K]_s$ accompanying a similar decrease in $[P]_s$ during the assimilation of glucose (11). There is a like decrease in these elements following the administration of insulin (14, 16) during the time that the concentration of glucose in the plasma is being reduced. Furthermore it should be remembered that epinephrine itself normally plays a rôle in carbohydrate metabolism in mobilizing glucose from the liver during exercise, insulin hypoglycemia and emotional crises.

It seemed possible that the rapid disappearance and after fall of potassium might be due in part to the effect of epinephrine. Accordingly the following series of experiments in which this point might be tested were planned.

Methods. 1. *Constant epinephrine in perfused hind limbs of frogs.* In order to maintain a blood supply having a constant [K] it was thought best to use the perfusion technique. Preparations of the isolated hind limbs of the double-pithed frog were used. The perfusion fluid was made up with the following composition: gum acacia 3.00 per cent; KCl, [redacted] .0450 per cent; $CaCl_2$, 0.050 per cent; glucose, 0.100 per cent; $NaHCO_3$, 0.068 per cent; Na_2HPO_4, 0.034 per cent; NaCl, 0.650 per cent. The pH was adjusted between 6.9 and 8.0. A perfusion pump (4) supplied pulsating pressure to the inflow cannula entering the terminal aorta. The outflow was collected from cannulae in the renal portal veins, all other egress being prevented by ligatures. [K] was determined (12) in arterial and venous samples collected at intervals during a period of $2\frac{1}{2}$ to $3\frac{1}{2}$ hours. Rates of flow were measured throughout. After a preliminary period of perfusion the fluid was changed to that containing 0.005 to 0.0025 mgm. epinephrine per cubic centimeter, and this was used throughout the remainder of the experiment (constant epinephrine). Two types of control

perfusion were done: one in which the perfusion was carried out as above except for the epinephrine (fast flow); the other in which the rate of flow was reduced to that found in the epinephrine perfusion, by reducing the perfusion pressure by half (slow flow).

The water lost by the perfusion fluid to the muscles was determined in a series of separate perfusions. The loss was determined on the bases of

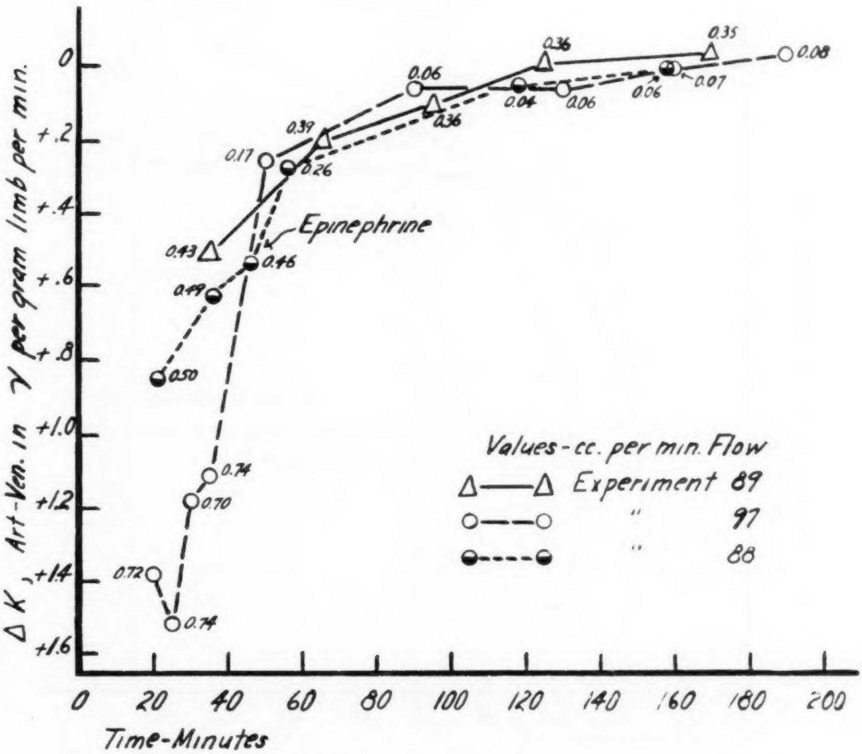

Fig. 1. Potassium exchanges in constant epinephrine (expt. 88), fast flow (expt. 89) and slow flow (expt. 97) perfusions of the hind limbs of the frog.

water contents of the fluid supplied to and the outflow from the hind limb preparations. In this series the water loss would not have caused an error of any significance.

2. *Potassium loss from stimulated frog muscles.* For these experiments the hind limbs of frogs were perfused with gum acacia-Ringer as described above. The muscles were stimulated indirectly by means of platinum electrodes in contact with the pelvic nerves in the abdomen. A Harvard

inductorium, the primary circuit of which was interrupted nine times a minute, was used.

In order to make certain that asphyxia or ischemia caused by low oxygen-content of the perfusion fluid were not factors two further experiments were carried out. In these the perfusion solution was approximately 4 per cent hemoglobin with the salt and pH content adjusted as before. The

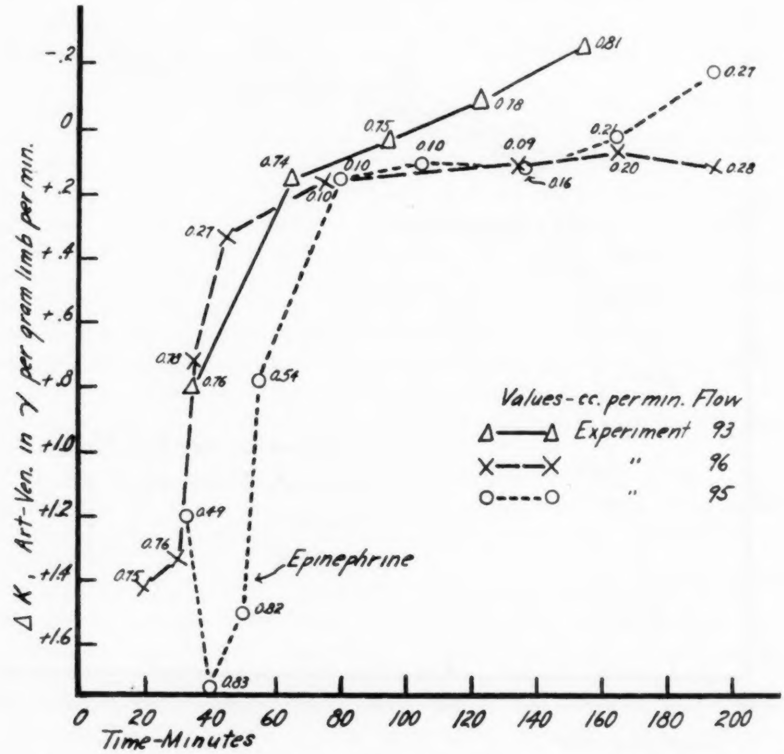

Fig. 2. Potassium exchanges in constant epinephrine (expt. 95), fast flow (expt. 93) and slow flow (expt. 96) perfusions of the hind limbs of the frog.

results were essentially the same as those obtained with the gum acacia-Ringer.

In two further stimulation experiments after a preliminary perfusion the perfusion fluid was changed to one containing 0.0025 mgm. epinephrine per cubic centimeter. The perfusion fluid was analyzed for potassium as before.

RESULTS. 1. *Constant epinephrine in perfused hind limbs of frogs.* The

results of five perfusions with constant epinephrine are shown in figures 1, 2 and 3. In the first two these are accompanied by the results of the fast flow and slow flow perfusions without epinephrine. The K exchange is shown graphically where the arteriovenous difference in gamma K per gram of perfused hind limb per minute is plotted against the time from the beginning of the perfusion. It will be noted that the [K] in the arterial in-

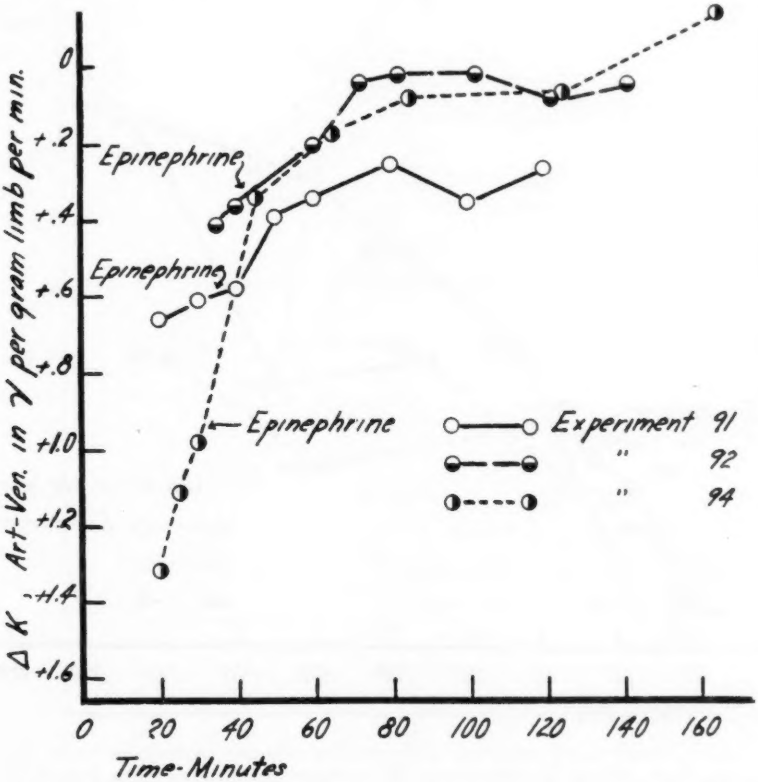

Fig. 3. Potassium exchanges in constant epinephrine perfusions of the hind limbs of the frog.

flow was the most important factor in determining the direction and rate of K exchange between the tissue and the perfusion fluid. The similarity of the decline of the rate of exchange under the three different conditions is very striking.

When the difference in [K] is plotted as in figure 4 it is evident that epinephrine has a marked effect on the arteriovenous [K] difference.

(Fig. 4 is based on the same experiment as fig. 1.) This effect, however, does not appear to be specific for epinephrine for the slow flow perfusion shows it as well.

2. *Potassium loss from stimulated frog muscles.* In the experiments on the stimulated frog muscles the customary uptake of K was soon reversed and a loss of K by the muscle became evident. The results are plotted in

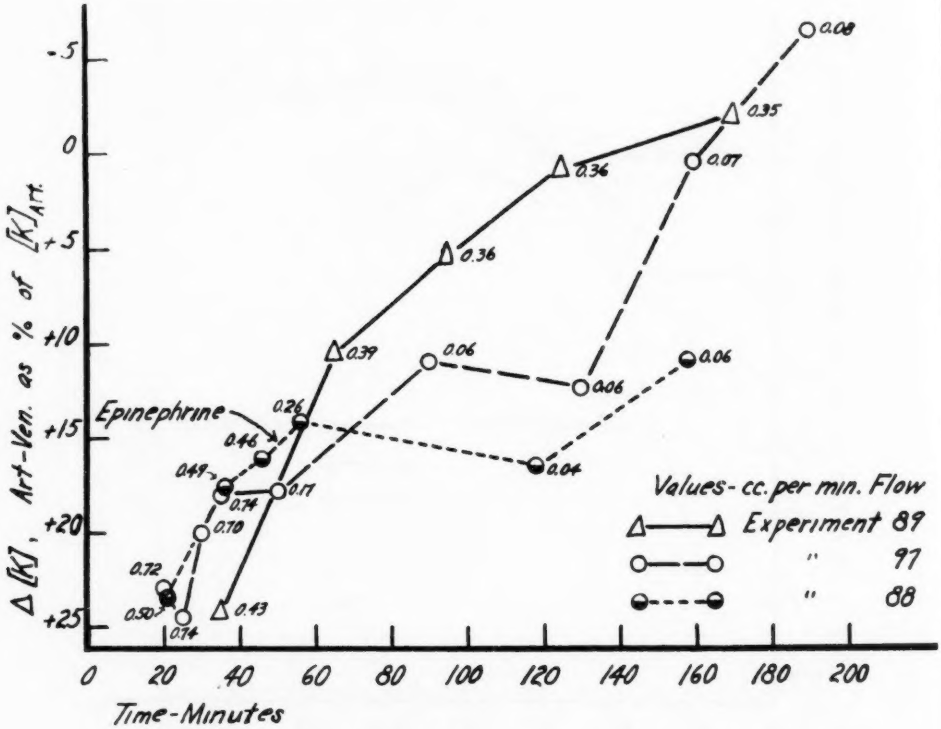

Fig. 4. Arteriovenous potassium differences in constant epinephrine (expt. 88), fast flow (expt. 89) and slow flow (expt. 97) perfusions of the hind limbs of the frog.

figure 5. The rate of K loss in both the fast flow and slow flow perfusions became quite constant at approximately 0.5 gamma K per gram of limb per minute. This rate of loss was maintained in each case until the experiments were terminated.

The effect of the constant perfusions with epinephrine in the two experiments in which it was used is also shown in figure 5. It will be noted that the K loss has been reduced in each case. The average magnitude of the reduction amounts to approximately 40 per cent.

DISCUSSION. The effect of the reduction in the blood flow upon the [K]$_s$ has been studied in dogs by Baetjer (3) and by Fenn (10). Their results, when recalculated to show actual exchanges, have been rather inconsistent, but have shown a reduction in the movement of potassium from tissue to

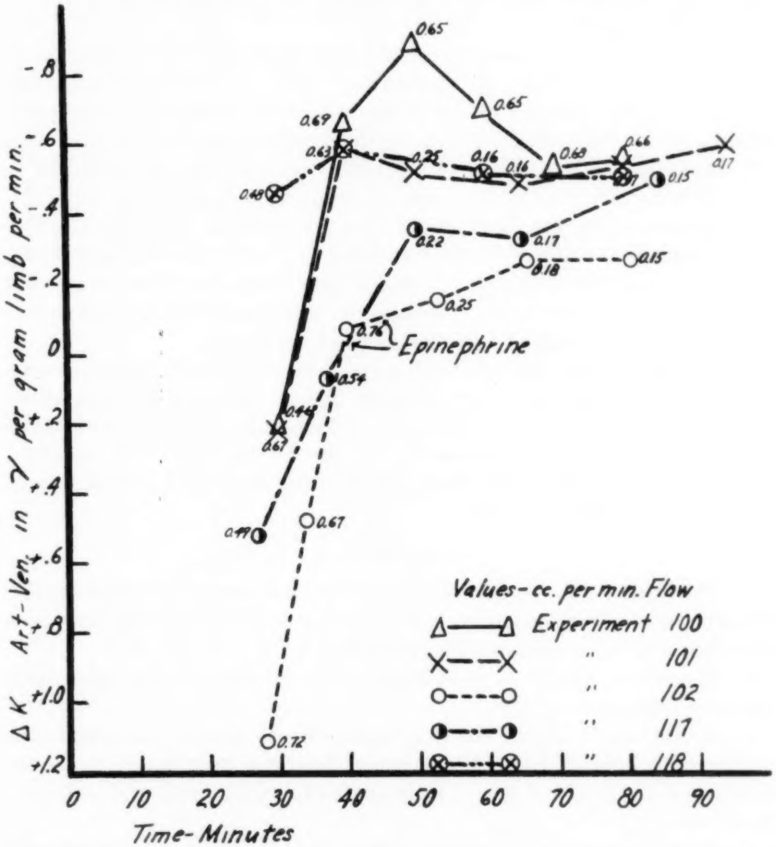

Fig. 5. Potassium exchanges in constant epinephrine (expts. 102 and 117), fast flow (expt. 100) and slow flow (expts. 101 and 118) perfusions of the hind limbs of the frog frequently stimulated.

vascular bed in most cases. These experiments were done on warm blooded animals where asphyxia due to a reduced blood supply might complicate the results.

On the frog where the question of asphyxia has been ruled out, as has been described above, the results have been quite uniform throughout.

It has been demonstrated that the exchange of potassium is independent of the total rate of renewal of the blood phase. This was true whether the rate of flow was altered by epinephrine administration or by simple change in the perfusion pressure. Furthermore, a direct effect of epinephrine upon the potassium exchange or balance in resting muscle has not been demonstrated.

The results of the perfusions of the hind limbs frequently stimulated confirm the work of Fenn (9) on the frog. Fenn reported an average loss of 2.6 mM. potassium per 100 grams dry weight of muscle after 90 minutes' stimulation. If one assumes that the dry weight of the muscles of the leg are one-tenth the wet weight of the entire limb, which was measured in the present study, one arrives at a figure for potassium loss of 1.27 mM. per 100 grams dry weight of muscle per 90 minutes' stimulation. This is approximately one-half the loss reported by Fenn. The difference may be due to a number of things. In the present study the perfusion was not continued beyond one hour. Perhaps the most important difference was that in the experiments of Fenn intact frogs were used.

The potassium sparing action of epinephrine in active muscles presents a new problem. Before a very thorough understanding of the significance of this can be reached the mechanism of the usual potassium loss in muscular activity must be accounted for. A further clarification of the effect of epinephrine on the other phases of muscular activity would also help in interpreting these results.

SUMMARY

1. The typical rise and after fall in the plasma potassium concentration following epinephrine injection have been confirmed in the dog and have been found to be present in sheep and goats.

2. The active uptake of potassium by the muscles in this connection has been confirmed on dogs.

3. In the hind limbs of the frog perfused with gum acacia-Ringer the uptake of potassium from the perfusion fluid has been found to be independent of the rate of flow. Epinephrine has not been found to have a direct effect.

4. The hind limbs of the frog similarly perfused and indirectly stimulated 9 times per minute have been found to lose potassium at the rate of 0.5 to 0.6 gamma of potassium per gram of hind limb per minute.

5. If a constant perfusion of epinephrine is used with the stimulated muscles it has been found to reduce the rate of potassium loss by 40 per cent.

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THE ACTIVITY OF HEMOLYSINS IN VIVO

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There have appeared in the literature, from time to time, statements regarding the existence of hemolysins normally present in the blood stream, descriptions of their nature, and methods for their extraction. Thus Brinkman (1922) obtained from blood a lysin which he identified with linolenic acid, and Bergheim and Fahraeus (1936) extracted from horse serum a lytic substance similar to lysolecithin. Bogaert (1937) also obtained a lysin from serum (human), but could not determined its nature. Ponder (1921), Miller-Lamb (1927) and Abels (1934), have shown that there is a lysin present in normal urine, and presumably it is also present in normal blood. Recently Freeman and Johnson (1940) have demonstrated the presence of lysins (soaps) in chyle, and the work of Wigodsky (1940) has raised a question as to the effectiveness of indol *in vivo*. Ellis and his co-workers have obtained lysins from a number of tissues, and Griffin (unpublished work) has extracted a potent lysin from the pancreas.

In order to be able to form an idea as to whether such substances can act as intravascular lysins and so contribute to red cell breakdown in the animal, it is necessary to know something about the relation between the activity of a lysin *in vivo* and its activity as tested in the usual *in vitro* systems containing washed red cells in low volume concentration (1–5 per cent). In general, the activity of a lysin is less *in vivo* than *in vitro* for two reasons: 1. The volume concentration of the red cells upon which it has to act is greater (30–40 per cent), and there are powerful inhibitors present in the blood stream in the form of the serum proteins. 2. Lysins *in vitro* can react only with the red cells of the system, but lysins *in vivo* can react not only with red cells, but with the cells lining the blood-vessels, etc.; in this way the effectiveness of the lysins, so far as the red cells are concerned, is reduced. As opposed to these, there is the fact that a lysin operating *in vivo* may be continually produced and its concentration maintained, whereas in an *in vitro* system the concentration falls continuously to a theoretical zero. The estimation of the relative effect of these various factors is the subject of this paper.

1. *The effect of cell concentration and of inhibitors.* Taken separately, the effect of variations in the concentration of cell suspensions has been

studied by Ponder (1930), and the effect of the presence of varying amounts of serum by Ponder (1923, 1925) and by Ponder and Gordon (1934). By an extrapolation from their data, some idea of the combined effect can be arrived at, but a direct experimental determination is more reliable, and this involves plotting time-dilution curves for hemolysis in whole blood. This again involves being able to measure the end-point corresponding to some given degree of lysis, e.g., 100 per cent hemolysis, and it was not until we tried to do it that we realized how difficult it is. Ordinary opacimetric methods cannot be used because of the great optical density of whole blood, and conductivity methods, which we tried to use, give poor results because of the large stirring errors associated with any considerable degree of lysis. Finally we found that the usual technique (Ponder, 1934) can be used if a piece of thin-walled glass tubing, of 3 mm. internal diameter, is placed in each of the tubes containing the hemolytic

TABLE 1

SYSTEMS	ρ	x, γ/cc.
Saponin, washed cells	0.006	15
Saponin, washed cells	0.10	298
Saponin, washed cells	0.35	1,060
Saponin, serum present	0.35	3,800
Na taurocholate, washed cells	0.005	125
Na taurocholate, serum present	0.36	30,400
Na oleate, washed cells	0.006	46
Na oleate, serum present	0.37	10,400

systems. By capillarity, the blood rises in the little tube, which is so narrow that the end-points corresponding to 100 per cent hemolysis can be easily determined. The little tubes can also be used to mix the systems.[1]

Proceeding in this way, we obtained results for the rabbit which are shown in table 1. This shows x, the quantity of lysin in γ/cc. required to produce complete lysis in 60 minutes in a number of typical systems. The volume concentration of the cells in each system is described by ρ.

The activity of a lysin such as saponin is only about $\frac{1}{250}$ as great in a system containing whole blood as in the usual test system containing washed cells in low concentration. For purposes of calculation one can say that the presence of serum and the high concentration of cells in whole blood reduces the activity of the lysin by a "factor of loss" of $\frac{15}{3800} = \frac{1}{250} = 4(10^{-3})$. For taurocholate the factor is $4.1(10^{-3})$, and for oleate it

[1] We have not been able to obtain satisfactory time-dilution curves with blood obtained from etherized animals. We kill the rabbit by a blow on the back of the neck, and then cut the carotids.

is $4.4(10^{-3})$. For human blood the factors will have a similar value (Ponder, 1934). It is sufficient for our purpose if the order of magnitude of the factor is determined.

2. *Uptake of lysins by tissues.* The extent to which lysins are taken up by tissues can be found with sufficient accuracy by perfusing organs, such as rabbit kidney and heart, with varying concentrations of the lysins in Ringer's solution, and determining the concentration of lysin which appears from time to time in the perfusate by means of a hemolytic titration.[2] The lysin is prepared in varying concentrations (from 1 in 2,500 to 1 in 40,000 in the case of saponin) in Ringer's solution, and is contained in a bottle connected with a cannula inserted in the renal artery or in the aorta. Another cannula is placed in the renal vein or in the right side of the heart, and from this samples of the perfusate are taken from time to time. A second bottle, connected with the arterial cannula by a T-tube with stopcocks, contains Ringer's solution.

The organ is first perfused with Ringer's solution in order to remove red cells and plasma; this may take from 15 minutes to several hours. After this, a sample of the perfusate is taken, and the small amount of inhibitory substances present is determined by a hemolytic titration. The organ is then perfused with the solution of lysin for several hours, the perfusion rate being continuously observed and kept as constant as possible; from time to time, small samples of the perfusate are collected in vials, and the concentration of lysin in each is subsequently determined by hemolytic titration.

Irrespective of the organ perfused (we have used kidney, heart, and the entire animal, and have done 62 of these perfusion experiments in all), the results are substantially the same. If we plot y, the fraction of the initial lysin concentration removed by the organ, against v, the volume of fluid which has passed through the organ, we get a fairly good straight

[2] A hemolytic titration is performed in the following way. A series of dilutions of the lysin, dissolved in Ringer's solution, are prepared, and systems of total volume 2 cc. are set up, containing 0.8 cc. of the various lysin dilutions, 0.8 cc. of Ringer's solution, and 0.4 cc. of a red cell suspension, prepared by suspending the thrice washed cells from 1 cc. of blood in 20 cc. of Ringer's solution. The mixture of lysin and Ringer's solution is brought to constant temperature in a water bath (described by Ponder, 1934), and the cells, also brought to constant temperature, are added. The time for complete hemolysis is measured, and if this is done for a number of dilutions of the lysin, a time-dilution curve results; this shows the relation between the dilution (or concentration in micrograms) of the lysin, and the time required for complete lysis. To find the lysin concentration in an unknown solution, such as a perfusate, one takes 0.8 cc. of the unknown, 0.8 cc. of Ringer's solution, and adds 0.4 cc. of the red cell suspension; the time for complete lysis is observed. Referring back to the time-dilution curve, this time corresponds to a time taken for complete lysis by a known concentration of the lysin; in this way the concentration of lysin in the unknown is immediately determined. The method is very precise, and can be modified in innumerable ways.

line in almost every case. The equation of this line is $y = b - bv/a$, and the total amount of lysin which the organ can take up is, by an extrapolation which will at least yield a *minimum* value, $U = C_o \cdot ab/2$ mgm. when C_o, the initial concentration of lysin, is measured in milligrams per cubic centimeter and when v is measured in cubic centimeters. The constant a is always a little less than unity, and b is the total volume in cubic centimeters which has passed through the organ when it has combined with so much lysin that it can combine with no more, i.e., when its lysin-binding powers are exhausted. The graph of the results of an experiment on rabbit kidney perfused with 0.5 mgm./cc. saponin will make these relations clear (fig. 1).

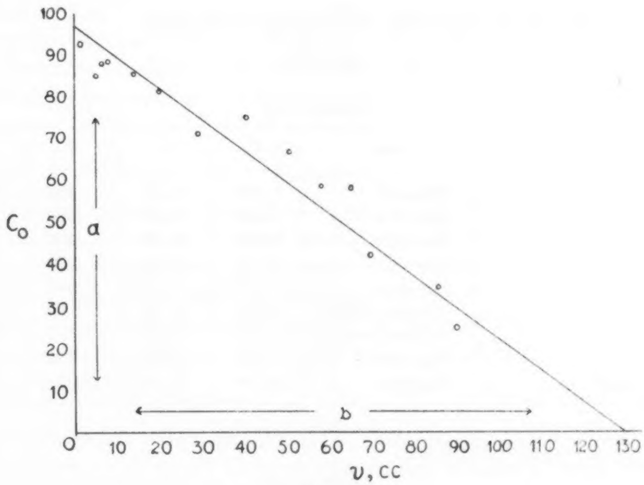

Fig. 1

The point of principal interest is the value of U, the total lysin-combining capacity of the organ, and table 2 shows some typical results in detail, together with the values of C_o, the initial concentration of lysin used in the perfusion fluid.[3]

In order to get an idea of the magnitude of the effects, take as typical the average of the values of U for the kidney perfused with saponin; this gives $U = 88$ mgm. per kidney or 13 mgm./gm. of kidney tissue. From table 1, the "uptake capacity," which must be of the same order as the amount of lysin required to produce complete hemolysis in an hour, is 3.8 mgm./cc. of blood, so, if we require to translate lytic activity in

[3] There may be a relation between C_o and U, but in our experiments it is obscured by variations in b, a constant which is affected by the perfusion rate. This again is affected by the very variable degree of vaso-constriction, and tissue breakdown, which the lysins produce.

whole blood into lytic activity for whole blood passing through kidney tissue, we have to introduce another "factor of loss" of about 3.4 (10^{-1}), because the tissues through which the blood flows have such a great affinity for the lysin, and enter into competition for it.

3. *Constant production.* The equation which at least formally describes the action of lysins *in vitro*, where an initial concentration of lysin c is established, and is used up in reacting with the cells, is

$$dx/dt = k(c - x)^n, \qquad (1)$$

whence, if $1/p = n$,

$$kt = \frac{p}{p-1}\{c^{\frac{p-1}{p}} - (c - x)^{\frac{p-1}{p}}\} \qquad (2)$$

and if $n = 1$, this becomes the well-known expression

$$kt = \log c/(c - x) \qquad (3)$$

TABLE 2

ORGAN	LYSIN	C_0	a	b	U
Kidney	Saponin	1.000	0.97	480	233.0
Kidney	Saponin	0.500	0.92	180	41.4
Kidney	Saponin	0.250	0.80	850	85.0
Kidney	Saponin	0.125	0.85	270	14.3
Kidney	Saponin	0.0625	0.75	2,840	66.6
Kidney	Taurocholate	2.500	1.00	40	50.0
Kidney	Glycocholate	2.500	1.00	105	131.0
Heart	Saponin	1.250	0.80	133	66.5
Entire rabbit	Saponin	0.250	0.95	6,640	789.0

If lysin is constantly supplied

$$dx/dt = kc \qquad (4)$$

and

$$kt = x/c \qquad (5)$$

To compare the effect of constantly supplying lysin as may occur *in vivo* (expressions 4, 5) with the effect of continually using up an initial quantity, as occurs *in vitro* (expressions 1, 2, 3), let us resort to a numerical example, using values for the constants which are generally found. Put $x = 20\gamma$ and $1/k = 200$ in (3); an initial concentration of 20.1γ will then bring about complete lysis in 1058 minutes. Put the same values in (5), and a constantly supplied quantity of 3.8 γ will produce the same effect in the same time. The constant supply accordingly gives a "factor of gain" in activity of about 5.3. Since 1058 minutes amount to about a day, we can take the value of x which produces lysis in infinite time in the ordinary test system (the asymptote of the standard time dilution curve),

divide it by a "factor of gain" of about 5.0, and so obtain the amount of lysin which, if constantly supplied, would produce the same amount of lysis in a similar system per day.

4. *Conclusion.* In considering the relation of lytic activity *in vivo* to that in the usual test system we have these three factors: $4(10^{-3})$ as a result of the cell concentration and presence of serum inhibitors in the former system, $3(10^{-1})$ as a result of the completion of tissues for the lysin, and -5.3 as a result of a constant supply of lysins *in vivo.* The result is a "factor of loss" of $\frac{1}{170}$ or 0.006. If we observe the activity of a lysin *in vitro* by means of the usual test systems, we have to think of its *in vivo* activity, as producing comparable effects, as about $\frac{1}{200}$ of that observed. This means that any lysin hitherto extracted from blood, urine or tissue, and the activity of which has been determined *in vitro,* would, *in vivo,* be a very weak lysin indeed. Whether such a weak *in vivo* lysin, given sufficient time, would have an appreciable effect on the state of the red cells of the intact animal is a matter beyond the immediate scope of this paper.

SUMMARY

The lytic activity of a simple lysin *in vivo* compared to that in the usual *in vitro* test system involves three factors: the effect of the high concentration of cells and presence of serum inhibitors *in vivo,* the fact that lysins are taken up by the tissues *in vivo,* and the fact that lysins may be constantly supplied *in vivo.* Taking these factors together, the lytic activity of a lysin in the whole animal is probably only about $\frac{1}{200}$ that observed in the usual *in vitro* test systems.

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THE EFFECTS OF DENERVATION ON PHOSPHOLIPID ACTIVITY OF SKELETAL MUSCLE AS MEASURED WITH RADIOACTIVE PHOSPHORUS

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As compared with tissues such as liver and small intestine, newly formed phospholipid makes its appearance very slowly in skeletal muscle. This has been shown with the aid of elaidic acid (1) and more recently with radioactive phosphorus (2, 3). In view of its bulk, however, muscle is an important depository of phospholipid. Thus it was found that about 36 per cent of the radioactive phosphorus incorporated into phospholipid by the whole bird appeared in relatively inactive tissues such as muscle, bone and blood within 12 hours after P^{32} injection (2).

It is shown in the present investigation that the normal rate of phospholipid activity of skeletal muscle depends upon its nerve supply. Denervation of skeletal muscle is followed by a profound increase in its content of labeled phospholipid synthesized in short intervals after the administration of radioactive phosphorus.

Experimental. The results recorded here were obtained from separate analyses of 144 rats, the data of part of which are shown in tables 1 to 5. Large adult rats, most of which weighed between 250 and 300 grams, were used. Denervation was performed by removing a small section of the sciatic nerve just below the trochanter while the rats were under light ether anesthesia. Radioactive phosphorus was injected subcutaneously as an isotonic solution of Na_2HPO_4; each rat received 1 cc. of this labeled phosphate solution. Muscles were removed at 2 intervals thereafter: 12 and 48 hours. Throughout this study analyses were made on a single group of muscles: the gastrocnemius and plantaris. These muscles can be rapidly removed together. Care was taken to ensure uniformity in sampling, the tendons and adhering tissues other than muscles being removed. The method by which the phospholipids were isolated and their radioactivity determined has been noted elsewhere (4). For the determination of total radioactive phosphorus, muscles were placed, with the usual precautions, in a muffle furnace maintained at 550° and the resulting ash transferred quantitatively by means of dilute HCl to blotting paper measuring 3 x 5 cm. The latter was dried over a hot plate,

wrapped in thin cellophane and its radioactivity determined in the manner described for phospholipid (4).

Results. *The content of labeled total phosphorus and of labeled phospholipid in corresponding normal muscles.* Before testing the effects of denervation, the uniformity of distribution of radioactive phosphorus and of radioactive phospholipid in similar muscles of the right and left legs of normal rats was determined. In table 1 the content of labeled phospholipid in the combined gastrocnemius and plantaris was compared in the right and left sides 48 hours after the subcutaneous injection of radioactive phosphorus. In 3 of the rats (168, 166 and 164) there was a close or fairly close agreement in the amount contained per gram of tissue in

TABLE 1

Phospholipid activity of corresponding normal muscles 48 hours after P^{32} administration*

RAT NO.	RIGHT LEG			LEFT LEG		
	Weight	Activity		Weight	Activity	
		Whole muscle × 10^2	Per gram × 10^2		Whole muscle × 10^2	Per gram × 10^2
	grams			*grams*		
160	0.95	0.68	0.71	0.91	0.87	0.96
161	1.13	0.65	0.58	1.11	0.73	0.66
162	1.35	0.87	0.64	1.36	1.07	0.79
163	1.54	0.74	0.42	1.44	0.97	0.60
164	1.27	0.49	0.39	1.26	0.48	0.38
165	1.43	0.96	0.67	1.31	0.99	0.76
166	1.23	0.92	0.74	1.28	0.97	0.70
167	1.55	0.99	0.64	1.53	0.82	0.54
168	1.36	0.90	0.66	1.38	1.02	0.73

* Measured as the per cent of the administered labeled phosphorus deposited as phospholipid.

both sides of the animal. Although differences were noted in the other animals, in no case did they exceed 42 per cent. The content of total labeled phosphorus in the combined gastrocnemius and plantaris is recorded in table 2. The values obtained per gram of tissue for right and left groups of muscles are in close agreement.

The effect of denervation on the content of labeled phospholipid of muscle. Radioactive phospholipid was measured in muscles at 2 intervals after injection of labeled phosphorus: 12 (table 3) and 48 hours (table 4). In the former, the muscles were examined at 12, 60, 108, 166, 204 and 300 hours after denervation. In muscles in which activity was determined 48 hours after P^{32} administration, denervation was performed 48, 120, 168, 216, 336 and 456 hours prior to their removal.

Twelve hours after denervation. In this group of rats (table 3) the labeled phosphorus was injected immediately after denervation and right and left muscle groups examined 12 hours later. In 4 of the 8 animals recorded, an increase of over 50 per cent in the labeled phospholipid content was observed in the denervated muscles as compared with those removed from the unoperated side. At this short interval after denervation, however, a rise in the content of labeled phospholipid did not consistently appear, for in 3 of the animals an insignificant change was observed after section of the sciatic nerve.

Forty-eight hours after devervation. Three rats received labeled phosphorus immediately after the sciatic nerve on one side had been sectioned and the phospholipid content of both gastrocnemii compared 48 hours later (table 4). At this early interval after denervation, it was again

TABLE 2

*Total radioactive phosphorus uptake of corresponding normal muscles**

RAT NO.	RIGHT LEG			LEFT LEG		
	Weight	Activity		Weight	Activity	
		Whole muscle	Per gram		Whole muscle	Per gram
	grams			*grams*		
97	1.33	0.19	0.14	1.39	0.17	0.12
98	1.39	0.19	0.14	1.40	0.20	0.14
99	1.52	0.22	0.14	1.55	0.19	0.13
100	1.73	0.18	0.10	1.75	0.17	0.10

* Measured as the per cent of the administered radioactive phosphorus. In this table the uptake of radioactive phosphorus 48 hours after its subcutaneous injection is recorded.

observed that although increases in labeled phospholipid were found in the 3 muscles whose sciatic nerves had been cut, the increase in only one of these could be regarded as significant.

Sixty hours after denervation. A striking increase in the content of labeled phospholipid was observed in the denervated muscles of 11 of the 12 rats recorded in table 3. Considerable variations were noted, but increases of over 100 per cent were obtained in 10 of the animals. The increase of *over 500 per cent* in a single animal is indeed worthy of note.

One hundred eight to three hundred thirty-six hours after denervation. In 35 animals the denervated muscles were compared with the intact muscles at 12 and 48 hours after the administration of P^{32}. The results recorded in tables 3 and 4 leave no doubt that increases in the labeled phospholipid content occur in muscles between the intervals of 108 and 336 hours after denervation. The increases for the *whole* muscle

TABLE 3

*Phosphololipid activity of muscles measured 12 hours after P^{32} administration**

TIME AFTER DENERVATION	RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
		Leg	Weight	Activity		Leg	Weight	Activity		Whole muscle	Per gram
				Whole muscle × 10^2	Per gram × 10^2			Whole muscle × 10^2	Per gram × 10^2		
hours			*grams*				*grams*				
12†	49	L	0.91	0.39	0.43	R	0.99	0.29	0.29	−26	−32
	50	R	1.11	0.26	0.23	L	1.17	0.26	0.22	0	0
	51	L	1.33	0.21	0.16	R	1.36	0.47	0.35	+120	+120
	52	R	0.75	0.17	0.23	L	0.72	0.32	0.44	+88	+91
	21	R	1.44	0.54	0.37	L	1.43	0.98	0.68	+81	+84
	22	R	1.77	0.38	0.21	L	1.78	0.81	0.45	+110	+110
	23	L	1.56	0.67	0.43	R	1.84	0.73	0.40	+9	+7
	24	L	1.57	0.40	0.26	R	1.51	0.57	0.38	+42	+46
60	25	L	1.98	0.20	0.10	R	1.85	1.22	0.66	+510	+560
	26	L	1.75	0.45	0.26	R	1.70	0.52	0.31	+16	+19
	27	R	1.57	0.30	0.10	L	1.53	0.85	0.56	+180	+460
	28	R	1.67	0.30	0.18	L	1.64	0.67	0.41	+120	+130
	9	R	1.02	0.36	0.35	L	0.97	1.32	1.36	+270	+290
	10	L	1.41	0.27	0.19	R	1.22	0.89	0.73	+230	+280
	11	R	1.31	0.43	0.33	L	1.27	0.65	0.51	+51	+55
	12	L	1.15	0.31	0.27	R	1.19	0.91	0.76	+190	+180
	5	R	1.81	0.47	0.26	L	1.73	1.14	0.66	+140	+150
	6	R	1.55	0.28	0.18	L	1.64	0.86	0.52	+210	+190
	7	L	1.70	0.27	0.16	R	1.57	0.78	0.50	+190	+210
	8	R	1.34	0.16	0.12	L	1.33	0.39	0.29	+140	+140
108	57	L	1.22	0.26	0.21	R	1.17	0.64	0.60	+150	+190
	58	R	1.07	0.52	0.49	L	0.93	0.82	0.88	+58	+80
	59	L	1.01	0.51	0.50	R	0.92	0.63	0.69	+24	+38
	60	R	1.06	0.37	0.35	L	0.89	1.06	1.30	+190	+270
166	37	R	1.60	0.27	0.17	L	1.56	0.45	0.29	+67	+71
	38	L	2.16	0.23	0.11	R	1.81	1.69	0.93	+630	+750
	39	L	1.45	0.34	0.23	R	1.19	1.51	1.27	+340	+450
	40	R	1.36	0.37	0.27	L	1.24	0.96	0.77	+160	+190
204	41	L	0.77	0.27	0.35	R	0.43	0.76	1.80	+180	+410
	42	R	1.10	0.24	0.22	L	0.89	0.71	0.80	+200	+260
	43	L	1.09	0.13	0.12	R	0.94	0.78	0.83	+500	+590
	44	R	1.09	0.11	0.10	L	0.91	0.36	0.40	+230	+300
300	45	R	1.20	0.17	0.14	L	0.91	0.43	0.47	+150	+240
	46	L	1.75	0.18	0.10	R	1.00	0.71	0.71	+290	+610
	47	L	1.17	0.38	0.32	R	0.85	0.92	1.10	+140	+250
	48	R	1.67	0.34	0.20	L	0.92	0.54	0.59	+59	+200

* Activity refers to the per cent of administered labeled phosphorus found as phospholipid.

† Phosphorus administered immediately after the nerves were cut.

TABLE 4

*Phospholipid activity of muscles measured 48 hours after P^{32} administration**

TIME AFTER DENERVATION	RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
		Leg	Weight	Activity		Leg	Weight	Activity		Whole muscle	Per gram
				Whole muscle $\times 10^2$	Per gram $\times 10^2$			Whole muscle $\times 10^2$	Per gram $\times 10^2$		
hours			*grams*				*grams*				
48	54	R	1.12	0.46	0.41	L	1.09	1.43	1.31	+210	+220
	55	L	1.09	1.45	1.33	R	1.08	1.61	1.49	+11	+12
	56	R	1.32	1.46	1.03	L	1.29	1.63	1.26	+12	+22
120	29	L	1.61	0.91	0.57	R	1.45	2.21	1.52	+140	+170
	30	R	1.67	0.22	0.73	L	1.46	2.25	1.54	+84	+110
	32	L	1.62	0.96	0.59	R	1.39	2.10	1.51	+120	+160
	65	L	1.32	0.99	0.75	R	1.05	1.91	1.82	+93	+140
	66	R	1.14	0.60	0.53	L	1.01	2.36	2.34	+290	+340
	67	R	1.03	0.66	0.64	L	0.84	1.21	1.44	+83	+130
	68	L	1.08	0.52	0.48	R	0.91	2.10	2.30	+300	+380
168	184	L	0.72	0.84	1.12	R	0.78	1.96	2.60	+130	+130
	185	R	1.20	1.48	1.23	L	0.81	2.31	2.85	+56	+130
	186	R	1.05	1.24	1.18	L	0.78	2.26	2.90	+82	+150
	187	L	1.27	1.46	1.15	R	0.92	1.80	1.96	+23	+70
	188	R	1.15	1.23	1.07	L	0.83	3.00	3.62	+140	+240
216	69	R	0.89	1.22	1.37	L	0.60	2.88	4.80	+140	+250
	70	L	1.10	1.03	0.94	R	0.81	2.69	3.32	+160	+250
	71	R	1.07	1.67	1.56	L	0.77	3.00	3.89	+80	+150
336	61	R	1.52	0.41	0.27	L	0.94	1.21	1.29	+200	+380
	62	L	1.57	0.56	0.36	R	0.88	1.25	1.42	+120	+290
	63	R	1.16	0.56	0.48	L	0.59	1.21	2.05	+120	+330
	64	L	1.40	0.53	0.38	R	0.74	1.27	1.72	+140	+350
456	172	L	1.38	0.75	0.54	R	0.52	1.36	2.62	+81	+380
	173	R	1.40	1.01	0.72	L	0.50	1.35	2.75	+34	+280
	174	R	1.41	0.72	0.51	L	0.37	0.84	2.27	+17	+340
	175	L	1.30	0.98	0.75	R	0.50	2.38	4.76	+140	+530
	176	R	1.42	0.73	0.51	L	0.52	2.10	4.04	+190	+690
	177	R	1.44	1.41	0.98	L	0.99	1.78	1.80	+26	+84
	178	L	1.06	0.76	0.72	R	0.42	1.76	4.19	+130	+480
	179	R	1.14	0.95	0.83	L	0.47	2.30	5.05	+140	+510
	180	R	1.27	0.86	0.67	L	0.53	1.44	2.71	+67	+300

* Activity refers to the per cent of administered labeled phosphorous found as phospholipid.

varied from 24 to 630 per cent in table 3 and from 23 to 300 per cent in table 4. Since at these intervals after denervation weight loss had already

occurred, the increases measured on the basis of per gram of tissue are even greater. Forty-eight hours after P^{32} injection (table 4) these increases were 70 to 380 per cent, whereas 12 hours after P^{32} administration (table 3) 38 to 750 per cent increases were observed per gram of muscle.

Four hundred fifty-six hours after denervation. The longest interval after section of the nerve in which comparisons of the phospholipid content of muscle were made was 456 hours (table 4). At this interval, the atrophy of the denervated muscle was quite marked. A consistent increase in the content of labeled phospholipid was still found in the denervated muscle, a difference that amounted to as much as 690 per cent when comparisons were made per gram of tissue.

TABLE 5

Total radioactive phosphorus uptake of corresponding denervated and intact muscles

TIME OF MUSCLE EXCISION		RAT NO.	INTACT MUSCLE				DENERVATED MUSCLE				PER CENT CHANGE IN DENERVATED MUSCLE	
After denervation	After P administration		Leg	Weight	Activity: Whole muscle	Activity: Per gram	Leg	Weight	Activity: Whole muscle	Activity: Per gram	Whole muscle	Per gram
				grams				*grams*				
60	12	89	R	1.36	0.18	0.13	L	1.31	0.23	0.18	+28	+39
		90	R	1.00	0.20	0.20	L	0.91	0.26	0.29	+30	+45
		91	R	0.99	0.18	0.19	L	0.94	0.22	0.23	+22	+21
		92	R	1.34	0.25	0.18	L	1.24	0.31	0.25	+24	+39
96	48	93	L	1.60	0.23	0.15	R		0.22		0	
		94	L	1.00	0.19	0.19	R	0.83	0.19	0.23	0	+21
		95	L	1.00	0.21	0.21	R	0.80	0.21	0.27	0	+29
144	48	101	L	1.30	0.21	0.16	R	1.03	0.27	0.26	+29	+63
		102	R	1.45	0.25	0.17	L	1.12	0.32	0.29	+28	+71
		103	R	1.24	0.28	0.23	L	0.94	0.27	0.29	+4	+26
		104	L	1.12	0.23	0.21	R	0.85	0.27	0.32	+17	+53

The effect of denervation on the content of total labeled phosphorus of muscle. In three groups of rats (table 5) the denervated and intact muscles were ashed and their total labeled phosphorus content determined. The time intervals following denervation at which muscles were excised were 60, 96 and 144 hours. The first group was injected with labeled Na_2HPO_4 12 hours before sampling, while in the latter two groups this time interval was 48 hours. As shown in table 5, a small but consistent increase in total labeled phosphorus appeared as a result of denervation when the muscles are compared per gram of tissue. In no case did this increase exceed 71 per cent. When whole muscles are compared with each other, the differences between denervated and intact muscles often become

negligible. The changes in total labeled phosphorus resulting from denervation in general are small compared to those obtained for labeled phospholipid in which increases of over 200 per cent often occurred.

DISCUSSION. Denervation markedly altered the rate at which skeletal muscle deposited newly formed phospholipid. When compared with the corresponding muscle of the intact side, the denervated muscle frequently showed *increases of over 200 per cent* in its content of radioactive phospholipid. This effect was not uniform at early intervals, but by the time 60 hours had elapsed after section of one of the sciatic nerves, a consistent difference in the radioactive phospholipid between the 2 muscles made its appearance. This change in the capacity of the denervated muscle to deposit labeled phospholipid appeared before appreciable atrophy set in and was present for as long as 19 days after section of the nerve. It should be noted here in passing that only small changes—an increase—in the water content of the muscle occur after denervation (5, 6).

The relation between total phospholipid content of a muscle and its *use* has been investigated by Bloor (7, 8, 9), who points out that the "more-used" muscles contain higher amounts of total phospholipid than "less-used" muscles. It would therefore appear at first sight that disuse of a muscle brought about by denervation would decrease its phospholipid content. Although this has been said to occur by Cahn (10), Grund (11) reports no change in the phospholipid content of a muscle as measured on the basis of the fat-free dry tissue. It should be stressed, however, that the loss of voluntary motor control is not the only effect of denervation (12); hence an alteration in total phospholipid content of a muscle (or for that matter in the rate at which radioactive phospholipid is deposited) need not necessarily reflect the effects of disuse.

SUMMARY

1. The phospholipid activities of normal and denervated muscles were compared, with radioactive phosphorus as indicator.

2. Denervation was followed by a pronounced increase in the capacity of the muscle to deposit labeled phospholipid.

3. This change made its appearance before appreciable atrophy of the muscle set in and was still observed 19 days after section of the nerve.

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THE EFFECT OF HYPERPYREXIA ON THE SECRETION AND FLOW OF BILE

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This investigation was undertaken to determine the effect of hyperpyrexia on the formation of bile. Many investigators (1, 2, 3, 4, 7) have used heat in various forms including localized diathermy over the region of the liver in the belief that heat so applied produces a choleresis. No conclusive evidence has been presented to support the thesis.

METHOD. Experiments have been performed on anesthetized dogs with a temporary fistula and on unanesthetized dogs with a permanent bile fistula.

Anesthetized animals. Thirty-nine healthy dogs weighing from thirty to forty-five pounds were used. The animals were placed on a standard diet and were not fed for 18 hours prior to the experiment. They were anesthetized with intravenous sodium pentobarbital using 30 mgm. per kilo of body weight. A tracheal cannula was inserted. The common bile duct was cannulated close to the duodenum, and a tube brought through the abdominal incision dropping the bile into a suitable vessel. The cystic duct was ligated and the edges of the abdominal incision were held in apposition. Bile flow was recorded electrically concurrently with carotid blood pressure. Once regularity of bile flow was established for a period not less than 30 minutes, a control flow was recorded. After a suitable control period of 20 or 30 minutes, heat was applied and maintained until the dog's rectal temperature rose to 42°C. The Inductotherm was used as a source of heat. The inductance cable or electrode fashioned in the form of an ellipse was placed on the animal board and covered with burlap material to form a suitable dielectric spacing between cable and dog. The dog was tied to the board in the usual supine position. The heating period usually lasted one hour after which the dog's temperature was permitted to return to normal. This usually required several hours even though an electric fan was used to assist in cooling the animal. The average number of drops of bile per minute covering a ten-minute interval during the control period was taken as the control rate. After the temperature of the animal had been elevated, the average number of drops

per minute during the 10-minute period showing the fastest flow was selected as the maximum rate of bile flow during pyrexia. The difference between this maximum and the basal control rate determined the rate of increase or decrease. The increase or decrease in the rate of bile flow, or minute volume output, was based on the number of cubic centimeters collected per minute. In this series we encountered two cases of acholia in which the liver would not even respond to intravenous sodium dehydrocholate.

Quantitative determinations for bile salts, cholesterol and bile pigment were made to ascertain whether or not there was a change in the biliary constituents. Biopsy of the liver was made before and after the application of heat in three animals.

Experiments on unanesthetized animals with a permanent bile fistula. Nine female dogs weighing between twenty and thirty pounds were used. Under ether anesthesia the cystic duct was cannulated, the gall bladder removed and the common bile duct ligated and cut. The rubber tubing from the cannula was coiled in the abdomen, led to the outside through a stab wound and attached to a rubber collecting bag. Following recovery from the operation, which usually required an average of ten days, the dogs were placed on a standard diet. The bile was collected and measured every 24 hours. When the quantity of bile was constant within ± 10 to 12 per cent, the animal was considered ready for treatment. The day prior to treatment bile samples were collected and measured at 8 a.m., 1 p.m., 3 p.m. and finally at 6 p.m. Collections were made and measured at similar time intervals during the experiment. Quantitative chemical determinations were made on the collected samples for cholic acid, bile pigment and cholesterol.

The dogs were placed in a cabinet which was heated by means of electric light bulbs. An electric fan kept the air in circulation. Air from the outside was introduced into the hot chamber through a rubber tube. The temperature of the box was kept at approximately 45°C. to 48°C. until the dog's rectal temperature reached 105° to 107°F., which was maintained for a period of one to two hours. This method of heating was chosen because of the difficulties encountered in using the high frequency current in heating dogs. During the experiment the dog could be observed through a glass window in the lid of the cabinet. Rectal temperature was taken with a Brown resistance recording thermometer.

Results. *Experiments on anesthetized dogs.* Of the thirty-nine dogs used, the flow of bile was recorded by a drop counter in ten, and by the drop counter and volumetric method in eleven; nine served as controls; in seven the skin was heated excessively; the remaining two were acholic and their livers were not even stimulated with bile salts given intravenously.

Table 1 gives the results of twenty-two experiments as recorded by the kymograph tracing. Subsequent to the heating period, the entire record showed an increase in bile flow. The maximum rate in most instances occurred just after the maximum temperature had been obtained (fig. 1). As a rule, the rate of bile flow did not show an increase until the rectal temperature reached 41.5°C. At this stage the dog usually began to pant. All of the twenty-one treated dogs showed an increased rate of flow over the basal rate. This increase ranged from 12 to 500 per cent.

TABLE 1

Showing the effect of artificial pyrexia on anesthetized dogs during the ten-minute period of maximum increase in bile flow

DOG NO.	RECTAL TEMP. °C.				AVERAGE BILE FLOW DROPS PER MINUTE			
	Initial	Max.	Rise	Final	Initial	Max.	Final	Increase per cent
1	37.7	42	4.3	39.3	2.2	3.3	1.7	50
3	38.2	42.5	4.3	38.0	1.4	2.2	1.0	51
4	39.9	42.5	2.6	39.8	1.4	2.1	1.5	50
5	37.8	42.0	4.2	37.4	3.5	5.1	3.0	45
9	38.6	42.0	3.4	39.0	1.1	1.6	0.59	50
10	37.8	42.3	4.5	38.8	3.2	3.6	2.1	12
12	39.0	42.0	3.0	39.5	1.9	2.8	1.3	47
14	38.5	42.0	3.5		2.5	3.6	1.7	44
15a	38.5	42.0	3.5	39.5	1.7	10.2	3.4	500
15b	39.5	42.0	2.5	41.0	3.4	5.0	3.2	300
16	38.5	42.0	3.5	39.0	2.4	6.3	2.5	162
19	38.0	42.0	4.0	39.5	5.0	8.2	4.2	64
20	38.0	42.0	4.0	39.2	3.7	4.8	4.8	30
21	37.2	42.0	4.8	39.0	1.7	5.0	2.0	194
22	37.5	42.0	4.5	38.2	1.4	7.3	6.1	421.3
24	37.8	41.5	3.7	38.3	1.6	2.9	2.2	81.2
25	39.1	42.1	3.0	40.7	1.7	3.6	1.7	111.6
26	40.0	42.6	2.6	39.5	2.2	2.7	1.1	22.7
27	.37.8	42.0	4.2	38.5	2.3	2.9	1.7	26.9
29	38.1	41.1	3.0	39.4	1.5	2.9	1.8	93.3
31	36.6	41.5	4.9	37.9	2.7	4.0	2.2	48.0
33	38.1	41.5	3.4	39.8	1.7	4.1	2.0	141.2

Dog 15a (table 1) showed a maximum increase of 500 per cent with a temperature elevation of 3.5°C. After cooling to normal, the animal was reheated 2.5°C. and this time the bile flow increased 300 per cent above the original control rate. All the dogs in which the skin was heated excessively with one exception either showed a decrease or else no change from the basal rate of flow.

During the course of the experiment, 5 to 10 hours, the bile flow frequently progressively decreased. This was shown by the results of the

control experiments. However, in 43 per cent of the treated animals the increased flow persisted after the temperature returned to its control level. The data in table 2 show that the average minute volume output increased in nine experiments from 13 to 100 per cent, whereas in two experiments a decrease in volume output occurred. The results of nine control experiments are also recorded in table 2.

Qualitative and quantitative chemical analysis of the bile was made on eleven treated and nine control animals. The summary of these data is included in table 2. Table 3 gives a typical protocol of an individual experiment.

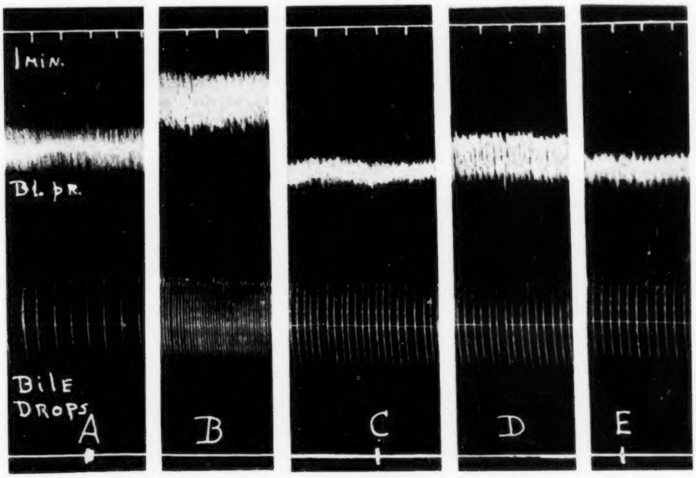

Fig. 1. Effect of hyperpyrexia on bile flow in a dog anesthetized with pentobarbital. *A*, control period; *B*, temperature 41.5°C.; *C*, temperature 39.0°C. three hours later than B; *D*, two hours after C; *E*, finish of experiment ten hours after control period.

We discovered that the rectal temperature of the control dogs did not remain constant under sodium pentobarbital anesthesia but continued to rise with time. This occurred even though an attempt was made to maintain basal temperature by the use of an electric fan. In seven of these dogs the temperature rose from 1° to 2.2°C. and was always maximal at the termination of the experiment.

The data on the chemical analysis of the bile for the treated animals show that a decreased minute output of cholic acid ranging from 13 to 70 per cent occurred in six animals while in three an increase occurred. Similarly, the cholic acid concentration was decreased in seven and increased in two. Five of the control dogs showed a decrease in the minute

output of cholic acid ranging from 10 to 64 per cent. The concentration of cholic acid was decreased in three, increased in one, and unchanged in one. In four of the control dogs we were unable to detect the presence of cholic acid in the last few specimens collected, although the analysis was repeated several times. Whether this was due to a cessation of the for-

TABLE 2

Quantitative and qualitative bile analysis

Eleven heated dogs, anesthetized

DOG NO.	TEMP. CHANGE	BILE VOL. (CC. MIN.)				CHOLATE OUTPUT (MGM. PER MIN.)			CHOLESTEROL OUTPUT (MGM. PER MIN.)			BILE PIGMENT OUTPUT (UNITS PER MIN.)		
		Total vol.	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change	Control	During and after heat	Per cent change
	°C.													
19	4.0	64.6	0.15	0.17	+13	3.31	2.34	−29.3						
20	4.0	40.9	0.14	0.12	−14.3	3.6	3.24	+10	0.018	0.025	+39			
21	4.8	32.3	0.07	0.10	+43	1.3	1.08	−16.9						
22	4.5	47.6	0.04	0.151	+77.5	1.23	2.49	+102.2				10.2	7.8	−23.6
24	3.7	23.8	0.055	0.088	+60	1.10	0.33	−70				1.3	1.1	−15.4
25	3.0	29.9	0.05	0.10	+100	1.44	1.60	+11.1	0.005	0.007	+40	6.78	9.0	+32.7
26	2.6	15.8	0.086	0.06	−30.2	0.87	0.43	−50.6	0.0017	0.0017	0	6.7	6.3	−6
27	4.2	26.4	0.058	0.08	+37.9	1.57	1.36	−13.4	0.0065	0.002	−70	5.7	5.6	−1.8
29	3.0	20.3	0.072	0.082	+13.7	2.6	1.14	−56.2	0.0090	0.0083	−8	1.68	5.2	+215
31	4.9	37.3	0.059	0.98	+37.9	Not secured			0.0079	0.0043	−45.5	5.7	4.7	−17.55
33	3.4	30.1	0.49	0.93	+89.7	Not secured						3.3	9.1	+175

Nine control experiments

DOG NO.	TEMP. CHANGE	TOTAL VOL.	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE	FIRST COLLECTION	SUBSEQUENT COLLECTIONS	PER CENT CHANGE
	°C.													
23	+2.2	35.2	0.044	0.087	+97.7	0.90	0.81	−10				5.4	7.3	+35
30	+1.4	15.6	0.050	0.040	−20.0	1.65	0.59	−64.3	0.0216	0.0067	−69	3.8	3.4	−10.6
32	+1.8	74.7	0.110	0.120	+9.1	Could not be read			0.0157	0.0068	−57	3.2	4.0	+25.0
34	+1.3	21.6	0.055	0.055	0	Could not be read			0.0035	0.0023	−34	6.2	8.8	+41.9
35	+1.7	5.6	0.023	0.018	−21.7	Could not be read						7.3	15.6	+113.0
36	+2.1	22.5	0.058	0.057	−1.7	Could not be read			0.0017	0.0015	−7	4.1	5.0	+22.0
37	+0.9	6.7	0.020	0.020	0	0.54	0.38	−29.6				11.8	16.1	+36.0
38	+0.6	6.9	0.025	0.017	−32.0	0.11	0.09	−18.0				1.5	3.8	+253.0
39	+0.2	48.5	0.130	0.110	−15.4	0.13	0.11	−15.3	0.0035	0.0023	−34	7.9	9.6	+21.0

mation of cholic acid by the liver or to a masking of the cholic acid test by large amounts of pigment could not be answered.

Four of the treated animals showed a decrease in the concentration of cholesterol while an increase occurred in two. The minute output of cholesterol was decreased in three, increased in two, and unchanged in

one. Five control dogs demonstrated diminished concentration and total cholesterol output.

Bile pigment determinations in the treated animals showed a decreased concentration and minute output in five and an increase in three. In eight of the controls, an increase in concentration and minute output of pigment occurred; a decrease occurred in one.

These results show that raising the body temperature of a dog anesthetized with pentobarbital from 3.0 to 4.9°C. increases the volume output of bile and when the results are compared with untreated controls usually decreases the output of bile pigment but has no significant effect on cholic acid and cholesterol output.

TABLE 3

Protocol of treated dog 22, anesthetized

BILE SAMPLE COLLECTION							BILE SALTS			CHOLESTEROL			BILE PIGMENTS	
Sample no.	Sample		Collected		Total cc. collec.	No. of cc. per minute	Mgm./cc.	Total	Mgm./min.	Mgm./cc.	Mgm. total	Mgm./min.	Units per cc.	Units per minute
	Time	Temp.	Time	Temp.										
	min.	*°C.*	*min.*	*°C.*										
Control	0	37.3	75	38.5	3.0	0.040	30.9	92.7	1.23				768	10.2
2	75	38.5	55	41.9	3.3	0.062	22.7	7.49	1.36				640	11.6
3	130	41.9	60	40.8	3.7	0.060	23.4	86.6	1.44				685	11.4
4	190	40.8	30	40.0	5.0	0.166	22.1	110.5	3.68				310	10.3
5	220	40.0	30	40.0	4.5	0.150	21.4	96.3	3.21				250	8.3
6	250	40.0	28	39.2	6.7	0.239	17.1	114.6	4.09	0.085	0.5695	0.0203	146	5.2
7	278	39.2	42	38.9	9.4	0.224	10.8	101.5	2.41	0.072	0.6848	0.0163	142	3.4
8	320	38.9	50	38.2	12.0	0.240	12.6	101.2	3.02	0.078	0.9360	0.0187	131	2.6
Total 6 hours 10 minutes					47.6			828.3					3072	
Average during and after heat period						0.151			2.49					7.8

Experiments on unanesthetized "chronic" bile-fistula dogs. Twelve experiments were performed on nine dogs. Only those experimental data are shown in table 4 in which following the treatment the animal ate all of the diet.

In interpreting the data on volume output no change that is not greater than ± 10 per cent is significant because that is the daily experimental variation.

The 24 hour *volume output* was increased significantly in two of the twelve tests and only one of these may be considered physiologically significant (dog 1). In seven of the twelve no significant change occurred. A significant decrease in volume output occurred in three of the twelve tests. The greatest decrease, 47 per cent, occurred in dog 8 during the second

TABLE 4

A summary of the effects of artificial pyrexia on chronic unanesthetized bile fistula dogs

DOG	24 HOUR VOLUME OUTPUT (CC.)			24 HOUR CHOLIC ACID (GRAMS)			24 HOUR PIGMENT OUTPUT (MGM.)			24 HOUR CHOLESTEROL OUTPUT (MGM.)			REMARKS
	Control	Heat	Per cent change	Control	Heat	Per cent change	Control	Heat	Per cent change	Control	Heat	Per cent change	
1	122	167	+37	1.20	1.19	Insig.	85.4	54.3	−36	9.12	14.84	+62.5	Diathermy
2	221	255	+15	2.83	2.63	Insig.	77.3	88.2	Insig.	No data			Diathermy
3	131	122	Insig.	1.26	1.87	+48	228.3	545.2	+139	7.49	8.31	Insig.	Hot box
4	141	132	Insig.	2.14	2.35	Insig.	173.9	176.8	Insig.	14.90	15.68	Insig.	In excellent condition throughout. Hot box
5	290	232	−20	2.07	2.27	Insig.	103.0	105.2	Insig.	21.20	19.57	Insig.	In excellent condition throughout. Hot box
6	160	147	Insig.	1.84	2.75	+49	70.3	75.7	Insig.	7.2	7.8	Insig.	In excellent condition throughout. Hot box
7(1)	171	148	Insig.	2.96	3.12	Insig.	109	173	+58.6	No data			In excellent condition throughout. Hot box
7(2)	188	188	0	3.92	2.97	Insig.	138	124	Insig.	14.6	11.5	Insig.	Local diathermy over region of liver. In excellent condition
8(1)	212	204	Insig.	2.56	3.84	+50	77.8	86.3	Insig.	7.14	7.09	Insig.	In good condition throughout. Hot box
8(2)	192	101	−47	2.56	1.17	−54	75.5	56.5	−25	7.65	4.26	−44	Local diathermy over region of liver. Skin heated excessively
9(1)	111	85	−23	2.31	1.24	−46	41.3	41.9	Insig.	3.18	2.31	Insig.	In good condition throughout. Hot box
9(2)	130	126	Insig.	2.56	1.71	−29	53.4	53.5	Insig.	7.58	5.70	Insig.	In good condition throughout. Hot box

Insig. = Insignificant—i.e., within range of normal control variation.

test in which the skin was heated excessively. *In no experiment during the period of artificial pyrexia was the flow of bile increased, which is in contrast with the results on the anesthetized animals.*

Significant increases in *cholic acid* output occurred in three of the twelve tests. A significant change did not occur in six tests. A significant decrease occurred in three tests, two of which were associated with a decrease in volume output.

No significant changes occurred in *cholesterol* output except in the animal whose skin was overheated. In two tests the *pigment output* was significantly increased, in two it was decreased, and no significant change occurred in the remainder.

These results show that during the presence of artificially induced pyrexia in unanesthetized dogs a decrease in volume output of bile occurs, and that the effect of a 2- to 3-hour period of pyrexia has a variable but no appreciable physiologically beneficial effect on bile formation.

DISCUSSION. Methods available for the study of bile secretion in the experimental animal and in man are not entirely satisfactory. Acute biliary fistula experiments in dogs introduce the factor of anesthesia and are limited to observations over short periods of time. Much information has been obtained from studies on chronic biliary fistula animals but this method is difficult and requires accurate control of the spontaneous variations which occur and which unless properly controlled may be large. Much of the available data in the literature is of questionable significance because of the failure properly to consider spontaneous variations. In man, the methods ordinarily employed are only roughly quantitative and often do not distinguish between true hepatic secretory effects and motor effects, unless a fully recovered patient with a total bile fistula is used.

In man, Goldbruger (1) reported an increased flow of dark bile from a duodenal tube during and after the application of diathermy. The increase in flow may well have been due to motor effects, especially since Rafsky (2) found that local diathermy over the liver region often appeared to cause a contraction of the gall bladder. Frisch and Lasch (3) report four experiments on two patients using a duodenal tube. Although they found some increase in volume output as well as in the bilirubin and cholesterol concentration following diathermy in one patient, the data are inadequate to permit a definite conclusion.

Couperus and Moore (4) applied local diathermy to the liver of trained chronic biliary fistula dogs and reported an increase of 7 to 17 per cent in the twenty-four hour bile volume output. Long observation of biliary fistula animals in this laboratory (5, 6) has shown that these animals on a standard regimen may show as much as ± 20 per cent daily variation in volume output when the bag technique is employed and about ± 8 per cent with the suction method (5). It may be that the increased output

reported by these authors was within the limits of normal variation, particularly since only one twenty-four-hour control period was recorded. These workers drained the bile by means of a tube in the fundus of the gall bladder. Hence variations in the concentrating and motor activities of the organ must be considered.

Reinhold and Wilson (7) obtained a mild choleresis by the application of external heat (60-W. electric lamp) over the region of the liver. This was observed in animals under anesthesia.

In the present study we have used both acute and chronic bile fistula animals. In the acute (anesthetized) animals, there was a definite increase in bile volume output in the treated group, which is in agreement with the observations of Reinhold and Wilson. It has been shown (8) that heat applied to the liver area of anesthetized dogs increases hepatic blood flow, and Tanturi and Ivy (9) have reported that an increase in hepatic blood flow increases bile secretion in acute experiments on anesthetized dogs. It is most probable that the increase in bile volume output observed in our anesthetized animals was the result of an increase in blood flow to the liver. In this connection it should be pointed out that dogs under sodium pentobarbital anesthesia do not begin to pant until a temperature of 41.5°C. is reached. Gibson and Kopp (10) have shown that the marked decrease in blood volume which occurs with external heating devices is not observed when no sweating occurs. Hence there should have been no significant reduction in blood volume in these animals, a factor which if it occurred would tend to counteract the influence of heat in increasing the hepatic blood flow.

The results of the bile chemistry studies on these anesthetized animals were too variable to permit any definite conclusions of special significance to be drawn.

The unanesthetized animals with a permanent bile fistula showed no significant increase in bile volume output over the control level. Although apparently contradictory to the "acute" experiments, this result should not be unexpected. Hemingway (11) has shown and we have confirmed that unanesthetized dogs begin to pant when their temperature is elevated only slightly and one would, therefore, expect a marked decrease in blood volume to occur in these animals (Gibson and Kopp). The work of Reinhold and Wilson (7) indicates that hydremia favors choleresis. Conversely anhydremia may diminish bile volume output.

It has been shown (9) that stimulation of the hepatic sympathetic nerves causes a marked decrease in bile secretion, presumably by hepatic vasoconstriction with a reduction in liver blood flow. The excitement which occurs even in a well-trained dog when its rectal temperature is raised to 106–107°F. might easily produce sufficient sympathetic stimulation to inhibit bile secretion. Hence we have two factors operating in the

unanesthetized animals which were not present in the dogs under anesthesia; namely, oligemia and sympathetic stimulation. Both tend to inhibit the secretion of bile. This would account for the suppression of the secretion of bile during hyperpyrexia and the variable results for the 24-hour secretion.

As in the anesthetized animals, the results of the chemical studies in the unanesthetized animals are too variable to be of any physiological significance.

The question now arises, what can be expected to occur clinically in man. From what has been said above, it is apparent that there are several factors concerned and the results cannot be predicted with certainty. Thus there may be either an increase in bile secretion, a decrease, or no effect depending upon the relative importance of the controlling factors in any given case. For example, in a relatively phlegmatic individual where measures are taken to avoid excessive fluid loss one might reasonably expect an increase in bile secretion. On the other hand, in a highly nervous and excited patient, a decrease might be observed.

SUMMARY AND CONCLUSIONS

1. A choleretic effect was produced in anesthetized biliary fistula dogs as a result of hyperpyrexia.
2. In unanesthetized biliary fistula dogs, a significant choleresis was not demonstrated.
3. The probable cause of these diverse results is discussed.
4. No significant effect of hyperpyrexia on the output of the various constituents of bile was observed.

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A REËXAMINATION OF THE RÔLE OF THE STOMACH IN THE DIGESTION OF CARBOHYDRATE AND PROTEIN

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It is generally taught that the stomach is of little or no importance in starch digestion and, at least by implication, that it plays a relatively important rôle in the digestion of protein. There are a number of considerations which cast some doubt on the validity of this concept. The frequency of symptomless achlorhydria renders it apparent that the body can dispense with the proteolytic activity of the stomach without demonstrable ill effects. In four patients with achylia pancreatica Beazell, Schmidt and Ivy (1) found that failure of protein digestion closely paralleled that of fat, although all of the patients secreted free acid and were presumably digesting protein in the stomach at a "normal" rate. None of these patients, on the other hand, manifested any gross failure in starch digestion, which suggests that hydrolysis by ptyalin might be of greater importance than it is usually considered to be. Thus, in the human, it is observed that deficient pancreatic digestion but "normal" gastric digestion results in a marked impairment of protein digestion and absorption, while starch is relatively well tolerated. In view of these observations it appeared desirable to reëxamine the rôle of the stomach in the digestion of protein and starch.

Very little direct *in vivo* data on gastric digestion are available. Many authors have used the rate of evacuation of the stomach as an index of the rate of gastric digestion (2). However, although gastric digestion and gastric evacuation are related, they are not necessarily mutually dependent. Total gastric digestion in the normal human subject can not be determined. Such a determination necessitates a duodenostomy or duodenal fistula from which the gastric discharge can be collected as it is being evacuated through the pylorus. The only satisfactory alternative is to withdraw the gastric contents by means of a tube. This only yields information on the state of digestion at the time the samples were obtained, but what has gone before and what might be expected to follow can be reasonably surmised.

Methods. In essence the experiment consisted of feeding normal

human subjects a meal of known nitrogen and carbohydrate content, withdrawing it from the stomach at the end of a specified period of time, and determining the ratio of "digested" to "undigested" nitrogen and carbohydrate in the recovered material. Since neither ptyalin nor pepsin characteristically reduce their respective substrates to absorbable products, the stage of degradation accepted as representing "digested" was chosen more or less arbitrarily. In the case of carbohydrate the production of reducing substances and in the case of protein the production of nitrogenous material soluble in 2.5 per cent tungstic acid were chosen as suitable end points of digestion. As used in this experiment, 2.5 per cent tungstic acid precipitates 47 per cent of the nitrogen in Bacto-pepton Difco (a commercial peptone preparation). Of the 53 per cent nitrogen remaining in solution only 22.8 per cent is in the form of ammonia and free amino acids. Thus, in addition to amino acids and ammonia, 2.5 per cent tungstic acid fails to precipitate many of the more complex products of protein digestion. Final proof that nitrogen soluble in 2.5 per cent tungstic acid constitutes a suitable end point for peptic digestion was obtained by an in vitro digestion experiment using U.S.P. scale pepsin. A 7.5 gram aliquot of the meal to be used was macerated, adjusted to pH 2.0, and digested with 100 mgm. of pepsin for 1 hour at 37°C. Under these conditions 37.3 per cent of the non-soluble nitrogen in the meal was digested to a form soluble in tungstic acid (preformed soluble nitrogen and that contributed by the pepsin being taken into consideration in the calculation). Throughout the following text nitrogenous material soluble in 2.5 per cent tungstic acid will be referred to as "soluble" nitrogen.

Eleven clinically normal young adult males were used as subjects for the experiment. In the initial phase of the study a test meal consisting of 150 grams of beef steak, 150 grams of potato, 50 grams of green peas, 10 grams of butter, and 200 cc. of water was employed. This meal was used in 2 experiments on each of two subjects who were capable of voluntarily regurgitating the gastric contents for analysis. The meal was masticated according to the normal habits of the subjects. Subsequently a finely divided homogeneous meal which could be completely evacuated from the stomach by means of an Ewald tube was employed. By resorting to the latter meal it was not only possible completely to evacuate the stomach (proven by x-ray control) but also to determine the dilution of the meal by gastric juice and saliva. As will be pointed out subsequently, this determination permitted interpretations which would not otherwise have been possible. The finely divided meal consisted of 100 grams of ground lean beef and 30 grams of potato starch boiled in sufficient water to yield a final volume of 400 cc. Twenty milligrams of phenolsulphonephthalein were dissolved in the water used in preparing the meal. The

dilution of the dye served to determine the dilution of the meal by the gastric juice and saliva.

The meal was ingested in the morning after a 14 hour fast. Either 30 minutes, 1 hour, or 2 hours later the gastric contents were obtained either by regurgitation or by means of an Ewald tube. The material recovered from the stomach was treated in the following manner. One sample was set aside for the determination of free and total acid (using dimethyl-amino-azo-benzene and phenolphthalein respectively as indicators) and pH (glass electrode). A second 30 cc. sample was added to a 5-minute old mixture of 15 cc. of 1.3 N sulphuric acid and 15 cc. of 10 per cent sodium tungstate. After thorough shaking the mixture was centrifuged and the supernatant fluid was further purified by filtration. This filtrate served for the determination of soluble nitrogen and reducing "sugar." A third sample was analysed for total nitrogen and a fourth for total carbohydrate. In all experiments the composition of the meal as well as of the material recovered from the stomach was determined. This was essential since the meal contained preformed reducing sugar and soluble nitrogen which had to be considered in determining the quantities of these substances produced in the stomach by digestion.

Nitrogen was determined by the macro Kjeldahl method and sugar by the hypo-iodite titration method (3). Total carbohydrate was determined by the A.O.A.C. method (4). Dilution was determined by the method of Wilhelmj et al. (5) as modified by Ivy, Schmidt and Beazell (6). All analyses were performed in duplicate.

The reliability of the dye method for determining dilution in the presence of a mixed meal was tested in vitro. It was found that a small, but measurable, quantity of the dye was adsorbed on the particulate material in the meal. Since the ratio of particulate material to fluid would not be the same in the material recovered from the stomach as it was in the meal, it must be concluded that, as used in this experiment, the dye method yields dilution values somewhat smaller than they should be. The error is not, however, of sufficient magnitude to confuse the interpretation in an experiment of this nature. In view of the recent observations of Hollander et al. (7), it is worthy of note that we have found tungstic acid precipitation entirely satisfactory as a method for clearing the material recovered from the stomach of interfering substances prior to developing the color of the dye.

Results. The results obtained with the unground meal are summarized in table 1. It should be emphasized that these results show only the state of digestion of the material remaining in the stomach. That which had been evacuated into the intestine was perhaps less extensively hydrolyzed. In this experiment it was not possible to calculate directly the quantity of digested material recovered from the stomach that repre-

sented preformed products of digestion in the meal. However, if it is assumed that the total carbohydrate and nitrogen left the stomach at the same rate as the "digested" carbohydrate and nitrogen, this factor can be taken into consideration in the calculation. The values in the table listed under the heading "per cent digested" were calculated on this basis. For example, if 10 per cent of the total nitrogen of the meal was in the form of soluble nitrogen, then it was assumed that 10 per cent of the total nitrogen recovered from the stomach was preformed soluble nitrogen, and in the calculation, this quantity was subtracted from the quantity of

TABLE 1

Gastric digestion of protein and carbohydrate normal meal

SUBJECT	MEAL						GASTRIC CONTENTS							
	Total nitrogen	Total soluble nitrogen*	Total carbohydrate	Total reducing sugar†	Per cent total nitrogen in form of soluble nitrogen	Per cent total carbohydrate in form of sugar	Volume recovered	pH	Total nitrogen	Total soluble nitrogen	Total carbohydrate	Total reducing sugar†	Per cent nitrogenous material digested in stomach‡	Per cent carbohydrate digested in stomach‡
1 hour period														
J. W.	5.6	0.84	42	7.56	15	18	175	4.5	1.73	0.175	6.3	4.13	0	47.7
H. A.	5.6	0.87	30.6	5.88	15.6	19.2	175	3.4	1.27	0.143	5.6	3.06	0	35.4
Ave.	5.6	0.86	36.3	6.72	15.3	18.6	175	4.0	1.50	0.159	5.9	3.59	0	41.5
0.5 hour period														
J. W.	6.75	0.90	34.3	4.85	13.3	14.2	215	5.1	2.12	0.200	8.6	4.98	0	43.8
H. A.	6.75	0.90	34.3	4.85	13.3	14.2	360	4.4	4.16	0.96	14.4	7.20	8.9	35.8
Ave.	6.75	0.90	34.3	4.85	13.3	14.2	287	4.7	3.14	0.58	11.5	6.09	4.5	39.8

* Soluble in 2.5 per cent tungstic acid.

† As maltose.

‡ Corrected for preformed digested material contributed by meal.

soluble nitrogen actually recovered from the stomach. The assumption that the "digested" and "undigested" fractions of the meal leave the stomach at the same rate is admittedly open to question. However, the error in interpretation would be greater if the factor of preformed "digested" material were neglected. Further, the digestion values compare favorably with those obtained in the subsequent experiments in which the preformed products of digestion in the material recovered from the stomach could be accurately determined (vide infra).

In only one of the 4 experiments of this series was the protein digested

to a measurable extent during the 30 or 60 minute period that it remained in the stomach. An important fraction of the carbohydrate, on the other hand, was hydrolyzed in every case. It is interesting to note that the digestion of the carbohydrate was not significantly further advanced at the end of 1 hour than it was at the end of 30 minutes. This might be anticipated since the activity of ptyalin diminishes rapidly as the pH falls.

The results obtained with the finely divided meal are summarized in table 2. Here again the values represent the state of digestion of the material remaining in the stomach at the end of 1 or 2 hours. Since in this experiment the stomach was completely evacuated and the dilution of the dye was determined, it was possible to calculate accurately the total quantity of the meal that had been evacuated into the intestine, and the fraction of the total digested material recovered from the stomach that represented predigested products in the meal. For example: if the meal contained 0.500 gram of soluble nitrogen and 50 per cent of the original meal was still present in the stomach at the end of the hour, then of the total quantity of soluble nitrogen recovered from the stomach, 0.250 gram would represent preformed soluble nitrogen in the meal. In a similar manner the theoretical starch content of the material recovered from the stomach was calculated.

Again in this experiment the quantity of nitrogenous material digested in 1 hour to products soluble in tungstic acid was insignificant (average 2.5 per cent) while an important fraction of the carbohydrate was digested to reducing sugar (19.6 per cent). When the period of time the meal was permitted to remain in the stomach was extended to 2 hours, the quantity of nitrogenous material "digested" was increased to an average of 9.5 per cent.

Discussion. On first examination the interpretation of these results appears to be open to criticism in the three respects listed below. It is felt, however, that all three criticisms can be convincingly refuted.

I. Since the optimum pH for pepsin activity is in the neighborhood of 2.0, it might be argued that a 1 hour digestion period does not permit the development of sufficient gastric acidity to fully activate the pepsin. However, it will be noted in table 2 that by the end of 1 hour, in all cases except two (H. W. and J. V.), more than 50 per cent of the nitrogen (average 69.1 per cent) had been evacuated from the stomach into the intestine. In other words, a meal of this type is largely evacuated from the stomach before significant protein digestion can occur. That subsequent more complete hydrolysis of the nitrogenous material remaining in the stomach at the end of an hour does occur is shown by the fact that when the digestion period was extended to 2 hours an average of 9.5 per cent was reduced to a soluble form. This value, representing the digestion of less than 50 per cent of the protein of the meal, would not support the concept that

the stomach is an important site of protein digestion. In the case of carbohydrate, on the other hand, maximum digestion occurs early (table 1), probably preceding the period at which gastric evacuation has assumed significant proportions.

TABLE 2

Gastric digestion of protein and carbohydrate finely divided meal

SUBJECT	VOLUME RECOVERED FROM STOMACH	VOLUME RECOVERED CORRECTED FOR DILUTION	ACIDITY			COMPOSITION OF MEAL		COMPOSITION OF MATERIAL RECOVERED FROM STOMACH					PER CENT STARCH DIGESTED TO SUGAR	PER CENT NITROGENOUS MATERIAL DIGESTED IN STOMACH	PER CENT INGESTED NITROGEN EVACUATED INTO INTESTINE
			Free	Total	pH	Total nitrogen	Total soluble nitrogen	Total nitrogen	Total soluble nitrogen*	Total sugar*	Starch equivalent of recovered sugar	Calculated starch of recovered material			
							1 hour period								
J. W.	230	197	22	48	2.6	3.04	0.34	1.23	0.037	4.27	4.00	14.8	27	3.0	59.8
R. M.	230	144	0	23	3.5	2.98	0.31	0.93	0	2.05	1.92	10.8	17.8	0	69.0
J. V.	350	292	11	52	2.8	2.45	0.34	1.79	0.070	4.16	3.80	21.9	17.6	3.9	27.0
G. L.	270	211	33	100	1.4	2.63	0.30	0.70	0.013	2.99	2.80	15.8	17.8	1.9	73.5
V. S.	215	176	30	84	1.7	2.63	0.30	0.64	0.002	2.35	2.20	13.4	14.4	0.3	75.8
H. W.	440	366	31	76	1.8	2.45	0.34	1.63	0.010	4.70	4.40	27.5	16.0	0.6	33.4
W. L.	340	188	16	55	2.5	2.39	0.30	0.71	0.047	4.21	3.93	14.1	27.8	6.6	70.0
M. B.	58	38	24	73	2.1	2.39	0.30	0.19	0.013	0.76	0.71	2.9	24.4	6.7	92.0
Ave.	267	202	21	64	2.3	2.62	0.32	0.98	0.024	3.19	2.98	15.2	19.6	2.45	62.5
							2 hour period								
J. W.	45	29.3	0	37	3.8	2.85	0.24	0.33	0.031	0.364	0.340	2.20	15.4	9.3	89
R. M.	100	79.0	21	56	1.9	3.26	0.27	0.28	0.018	0.387	0.360	5.90	6.1	6.5	92
H. W.	130	45.8	25	72	1.8	2.80	0.25	0.22	0.025	0.465	0.435	3.45	12.6	12.1	92
J. C.	140	140.0	35	108	2.0	3.32	0.28	0.83	0.086	1.960	1.830	10.5	17.5	10.3	75
T. K.	100	72.8	30	92	1.7	3.32	0.28	0.28	0.024	0.940	0.880	5.47	16.1	8.8	92
Ave.	103	73.4	22.5	73.0	2.2	3.11	0.26	0.39	0.037	0.823	0.769	5.50	13.5	9.5	87.5

* Corrected for preformed digested material contributed by meal.

II. It might also be argued that the stomach would selectively evacuate the products of digestion leaving behind only the undigested residue. However, the soluble products of digestion would leave the stomach no more rapidly than the equally soluble dye and, in the second series of experiments, all interpretations are influenced by the quantity of dye remaining in the stomach at the end of the 1 hour period.

III. It is generally accepted (8) that protein hydrolysis by pepsin does

not proceed to a significant extent beyond the production of peptone. Therefore nitrogen soluble in tungstic acid (which precipitates approximately 50 per cent of the nitrogen in a relatively pure peptone preparation) might be considered too far advanced in the hydrolytic series to represent a suitable end point of pepsin hydrolysis. However, it was shown (vide supra) that in a period of 1 hour under optimal conditions, pepsin is capable of reducing 37 per cent of the nitrogen of the standard meal to a form that is soluble in tungstic acid. It might be considered, then, that although pepsin is potentially a potent proteolytic agent, conditions in the stomach are not such as to permit it to operate effectively. Further, so far as comparing the relative importance of protein and starch digestion is concerned, reducing sugars are further advanced in starch hydrolysis than tungstic acid soluble nitrogen is in protein hydrolysis.

Since, during the period the meal remained in the stomach before being withdrawn for analysis the extent of the digestion of that fraction which was evacuated into the intestine was undetermined, the total quantity of starch and protein digested can not be calculated; i.e., as has been noted previously, only the state of digestion of the material remaining in the stomach is known. However, minimum values for the quantity of both carbohydrate and protein digested can be calculated. In the case of the 1 hour digestion period with the finely divided meal, 50.5 per cent of the carbohydrate, or 15.2 grams, and 37.5 per cent of the nitrogen, or 6.08 grams as protein (0.98×6.2) remained in the stomach. Of these quantities, 2.98 grams of carbohydrate had been digested to reducing sugars (expressed as maltose) and 0.149 gram of protein had been digested to a soluble form. Thus it can be said that at least 9.9 per cent (2.98/30) of the carbohydrate and only 0.92 per cent (0.149/16.3) of the protein is digested to the prescribed end point when a meal of this nature is ingested by the average normal human subject.

It should not be concluded that, on the basis of these observations, the author is dismissing the stomach entirely as a site of protein hydrolysis and concluding that it is indispensable in the digestion of starch. The factor of safety in digestion is unquestionably great, and in man as in the dog (9) pancreatic amylase would be sufficiently abundant to hydrolyze any reasonable quantity of starch in the complete absence of ptyalin. It should be remembered, however, that disturbed nutrition is not the only way in which digestive inadequacies may be manifested.

The fact that only 2.5 per cent of the "nitrogen" was reduced to a soluble form in 1 hour does not, of course, mean that the remaining 97.5 per cent was completely undigested. In the hydrolysis of complex substances such as proteins and starches the earliest cleavage products are a mixture of complex and simple substances, with the former predominating. As

digestion proceeds there is an increasingly greater rate of formation of the ultimate hydrolytic products. Unquestionably a large fraction of the protein had been partially digested, although not to a tungstic acid soluble form. It should be noted, however, that in all cases the material recovered from the stomach contained appreciable quantities of readily recognizable meat, but no native starch as indicated by the iodine test.

On the basis of these results, there appears to be ample justification for the conclusion that, in the past, in considering the importance of the human stomach in digestion, the emphasis has generally been misplaced. It would appear that the importance of the stomach in the hydrolysis of starch, as a result of the action of ptyalin, has not been fully appreciated while its importance in the hydrolysis of protein has been over emphasized.

SUMMARY

Using young normal male adults as subjects, the relative importance of the human stomach in the digestion of protein and starch has been investigated. The experiment consisted of feeding the subjects a meal of known composition, removing it from the stomach at the end of a specified period of time, and determining the ratio of "digested" to "undigested" protein and starch in the material recovered from the stomach. The production of reducing sugars in the case of starch, and the production of nitrogenous material soluble in 2.5 per cent tungstic acid in the case of protein, were arbitrarily selected as end points of digestion.

In 4 experiments in which the test meal corresponded with what might be considered an "average" meal, samples for analysis were obtained by having the subjects empty their stomach as completely as possible by voluntary regurgitation at the end of either 0.5 or 1 hour. Under these circumstances it was found that, irrespective of the time that the meal remained in the stomach, approximately 40 per cent of the remaining carbohydrate was in the form of reducing sugars, while essentially none of the protein had been reduced to a form soluble in tungstic acid.

In 13 experiments in which a finely divided meal was used, the stomach was completely evacuated at the end of either 1 or 2 hours by means of an Ewald tube. Under these circumstances it was found that of the material remaining in the stomach at the end of 1 hour, an average of 19.6 per cent of the starch was in the form of reducing sugars while only 2.45 per cent of the nitrogen was soluble in tungstic acid. When the digestion period was extended to 2 hours, 9.5 per cent of the nitrogen remaining in the stomach was in a "digested" form.

In view of these observations it is felt that the conventional concept that the stomach plays an unimportant rôle in the digestion of starch and an important rôle in the digestion of protein, is open to revision.

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THE EFFECT OF HISTAMINASE ON THE GASTRIC SECRETORY RESPONSE TO HISTAMINE[1]

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In a previous study we (1) found that histaminase given subcutaneously and slowly intravenously failed to influence the gastric secretory response to histamine and to a meal. Since then reports have appeared indicating that histaminase given orally or intramuscularly may possess therapeutic value in allergic conditions (2) and may depress the gastric secretory response to histamine and to a cold bath (3).

This work was undertaken to study the effect of histaminase, given intramuscularly to dogs and orally to human subjects, on the gastric secretory response to histamine. Such work was not included in our previous study because the negative results we obtained were theoretically predictable on the basis of a consideration of some of the *in vitro* observations of Best and McHenry (4), who for similar reasons have expressed doubt regarding the therapeutic promise of histaminase (5).

The histaminase powder used. The kidney histaminase used was obtained through the courtesy of the Winthrop Chemical Company. All lots were assayed by us a few days prior to their use. Fifteen milligrams of the powder were found to contain one unit of histaminase. We also made a preparation of histaminase from dog's intestinal mucosa; it contained one unit of histaminase in either 30 or 45 mgm. depending on the lot, and no histamine.

Experiments and results. *Heidenhain pouch dog.* After collecting the continuous secretion for three one-half-hour periods, 0.25 mgm. histamine dihydrochloride was injected subcutaneously and 10 minutes later the injection was repeated. The secretion was collected at 10 minute intervals for one hour, when the response to histamine had about disappeared. The total volume of secretion for one hour was measured. The histaminase was then injected intramuscularly and 25 or 30 minutes later histamine was injected as before. This time interval between the histaminase and histamine injections was chosen because it is one reported to be effective (3).

[1] This research was aided by a grant from the Marjorie Newman Research Fund.

Eight units of kidney histaminase were injected in 7 experiments in a Heidenhain pouch dog on different days. The results are summarized in table 1. It is to be noted that on the average the secretion of acid was slightly reduced. Since the response of an animal to histamine varies from day to day and even to the same dose given 1.5 or 2 hours apart, it is necessary to test the significance of the differences observed by statistical methods. When the data are statistically analyzed, the difference in

TABLE 1

Showing the summarized results before and after histaminase given intramuscularly on the gastric secretory response to histamine (0.25 mgm.) in dogs

TYPE OF ANIMAL PREPARATIONS AND DOSE OF HISTAMINASE	NUMBER OF TESTS	VOLUME OUTPUT OF JUICE		OUTPUT OF HCl		REMARKS
		Before	After	Before	After	
		cc.	*cc.*	*mgm.*	*mgm.*	
Heidenhain pouch. 8 units of kidney histaminase	7	7.4 ±0.4*	6.9 ±0.6*	20.3 ±1.4	16.0 ±1.9	The difference in HCl output is 4.3 ± 2.3 and is not significant
Heidenhain pouch. 15 units intestinal histaminase	7	8.2 ±1.0	8.3 ±1.0	30.9 ±5	29.3 ±4	The lack of a difference is obvious
Total gastric pouch. Vagi cut. 30 units kidney histaminase	12	85.4 ±9.3	76.3 ±9.3	410 ±45	358 ±40	The difference in HCl output is 52 ± 50 and is not significant
Total gastric pouch. Vagi cut. 30 units inactivated kidney histaminase	8	80.2 ±7.4	67.7 ±8.6	406 ±40	324 ±45	C.R. of volume output is 1.29 and of acid output 1.06. The difference is insignificant

* Standard error of mean.

the volume and acid output before and after histaminase is found not to be significant.

Believing that a larger dose of histaminase might yield significant differences, 15 units of intestinal histaminase were injected in 7 experiments, the experimental procedure being the same as in the foregoing group of experiments. A significant difference was not observed. The results are summarized in table 1.

Total gastric pouch dog, with vagi cut. Thirty units of kidney histaminase were injected intramuscularly in 12 experiments. The experimental procedure and the dose of histamine were the same as in the experiments on

the Heidenhain pouch dogs, except the secretion was collected for seven instead of six 10 minute periods.

The volume and acid output appeared to be decreased after histaminase. It was decreased in 7 of the 12 experiments, increased in 3 and no change occurred in 2. When the differences were analyzed statistically, no significant change was found.

In view of these results it seemed unnecessary to conduct controls with *inactivated histaminase*. This was done, however, in 8 experiments to be certain that the histaminase powder contained no gastric secretory excitant other than histamine, the presence of which was ruled out by blood pressure assay. If it should contain such a secretory excitant, it would mask the effect of histaminase. This was not likely because the injection of the active histaminase powder was never observed definitely to stimulate. It is possible, however, that the tendency to diminished secretion that occurred in 7 of the 12 tests might be due to the injection of impurities present in the histaminase powder. The histaminase preparation was heated at 60°C. for 20 minutes to inactivate the enzyme.

The results of the intramuscular injection of inactivated histaminase are shown in table 1, and are almost identical with those obtained when the active preparation was administered.

Human subjects. Histaminase administered intraduodenally. Sixteen human subjects who were accustomed to swallowing a stomach tube were used. A double tube was swallowed, one of which passed about 10 inches into the duodenum and the other remained in the stomach. Alkaline fluid containing bile from the duodenal tube and acid fluid containing no bile from the gastric tube, showed that the tubes were in proper position. These criteria were required in all tests. After the double tube was in place the stomach was emptied of its residual fluid and the continuous secretion was collected for three 10 minute periods, saliva being expectorated. Then 0.5 mgm. of histamine dihydrochloride was injected subcutaneously and the secretion collected continuously, measured and titrated every 10 minutes for one hour and 10 minutes. Then, 100 units of kidney histaminase (1.5 grams) powder suspended in 30 cc. of 0.9 per cent NaCl solution were introduced into the duodenum through the duodenal tube, the tube being washed with 10 cc. of water. The duodenal tube was then clamped. The collection of gastric juice was continued. Twenty minutes after the introduction of the histaminase, 0.5 mgm. of histamine was injected and the gastric juice collected for 1 hour and 10 minutes.

In the first six subjects the test was performed only once. In the ten subjects who followed, the test was repeated about one week later.

The summarized results are shown in table 2. The histaminase obviously had no effect. The volume output of juice for each 10 minute period is shown in figure 1. A curve, curve *C*, showing the volume output

of juice of 20 subjects in 71 tests in response to 0.5 mgm. of histamine dihydrochloride is included in the figure.

Human subjects. Histamine administered orally for three days. Since histaminase is thought to be active when given orally in enteric coated

TABLE 2

Showing the response of 16 human subjects to histamine (0.5 mgm.) before and after the introduction of histaminase (100 cc.) into the duodenum

SUBJECT NUMBER	VOLUME OUTPUT FOR 1 HR. 10 MIN.				OUTPUT OF HCl FOR 1 HR. 10 MIN.			
	Before		After		Before		After	
	Average	Each test	Average	Each test	Average	Each test	Average	Each test
	cc.	*cc.*	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	88		120		170		227	
2	101		58		343		188	
3	90		91		157		178	
4	133		195		513		743	
5	156		146		706		694	
6	86		121		339		364	
		101.5		109		489		497
7	99	97.0	102	95	443	396	415	332
		184		125		731		322
8	172	160	154	183	672	611	462	602
		100		102		256		257
9	119	138	115	127	308	360	306	354
		130		162		578		624
10	137	144	155	148	613	647	560	495
		97		95		380		353
11	99	101	92	90	362	344	358	362
		108		113		388		398
12	116	129	103	94	426	465	368	337
		70		221		164		472
13	69	68	197	172	150	136	549	525
		131		128		437		381
14	120	108	115	102	408	379	342	302
		292		329		1200		1347
15*	270	249	352	375	1026	852	1139	931
		325		298		1308		1062
16*	276	227	265	233	1057	808	941	819
Average	133		149		481		490	

* The subjects had an active duodenal ulcer.

tablets, it is possible that the repeated administration of histaminase might be effective although a single administration might not be.

Accordingly, 8 subjects that responded quite consistently to histamine were given 150 units of histaminase daily for three days. Fifty units were taken in the form of enteric coated tablets (these were assayed and proved

to be active) one-half hour before meals. On the morning of the fourth day tablets containing 50 units were taken with some water one-half hour prior to the passage of the stomach tube. The histamine test was then performed as had been done prior to the three-day histaminase period.

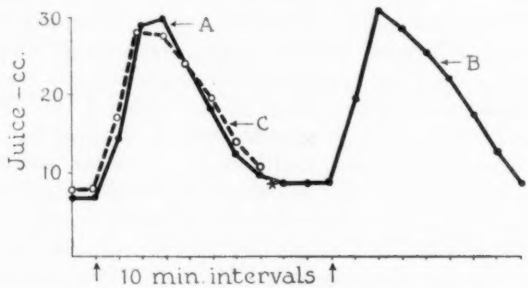

Fig. 1. Volume in cubic centimeters. Response to 0.5 mgm. histamine dihydrochloride.

——*A* = Before histaminase—26 tests on 16 subjects; 10 subjects tested twice.

——*B* = 25 minutes after histaminase.

-----*C* = Response of 20 subjects in 71 tests.

Output HCl in 1 hour, 10 minutes. *A*, 481 mgm.; *B*, 490.

Volume output, *C* = 140 cc.; HCl output 485 mgm.

* Histaminase, 100 units into duodenum.

TABLE 3

Showing the response of 8 human subjects to histamine before and after taking 150 units of histaminase in the form of enteric coated tablets for 3 days

SUBJECT NUMBER	VOLUME OUTPUT FOR 1 HR. 10 MIN.		OUTPUT OF HCl IN 1 HR. 10 MIN.	
	Before	After	Before	After
	cc.	*cc.*	*mgm.*	*mgm.*
1	172	180	672	752
2	119	121	308	334
3	137	166	613	521
4	99	90	362	220
5	99	116	443	521
6	179	208	631	669
7	107	117	416	447
8	80	78	134	136
Average..........	124	134	447	451

The results are shown in table 3. The histaminase had no effect on the response to histamine.

The data of five of these subjects on each of whom from 5 to 8 histamine (0.5 mgm.) tests had been performed were analyzed for variability to obtain some idea regarding the extent to which the response of the individual

varies from test to test. The subject manifesting the minimum variability secreted 93 cc. ± 5 cc. (S.E.) and 290 mgm. HCl ± 50 mgm. (S.E.). The subject manifesting the maximum variability secreted 166 cc. ± 14 and 602 mgm. HCl ± 87 mgm.

DISCUSSION. The failure of orally administered histaminase to decrease the gastric secretory response to histamine is not surprising in view of its inactivation by HCl-pepsin and by tryptic digestion (5) and of the fact that it has never been demonstrated that active enzymes are absorbed in significant amounts from the lumen of the intestine (6). Neither is it surprising that intramuscularly administered histaminase has no effect on the gastric secretory response to histamine, since we found previously that the continuous intravenous injection of histaminase during the absorption of histamine from subcutaneous tissue is ineffective (1).

Attention should be called to the fact that the response of the stomach to a standard dose of histamine is not constant. Hence, when the effect of a substance on gastric secretion is being studied, the significance of differences between averages should be examined by the statistical method.

Therapeutic studies of histaminase action to date are characterized by a failure to use inactivated histaminase as a control. Such a control would seem to be essential, particularly in view of the fact that all preparations of histaminase are very impure.

CONCLUSIONS

1. From 8 to 15 units of histaminase injected intramuscularly in a Heidenhain pouch dog and 30 units similarly injected into a dog with a pouch of the entire stomach failed to decrease the gastric secretory response to 0.5 mgm. of histamine dihydrochloride.
2. One hundred units of histaminase introduced into the duodenum 20 minutes before the injection of 0.5 mgm. of histamine failed to decrease the gastric secretory response in a group of 16 human subjects.
3. The administration of 50 units of histaminase in the form of enteric coated tablets three times daily for three days to 8 human subjects had no effect on their gastric secretory response to 0.5 mgm. of histamine.

In this and a preceding study we have been unable to demonstrate that histaminase given by any route has a significant inhibitory effect on the gastric secretory response to 0.5 mgm. of histamine.

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THE EFFECTS OF DIRECT CURRENTS UPON THE ELECTRICAL EXCITABILITY OF NERVE

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Previous observations made in this laboratory (Reboul and Rosenblueth, 1939; Rosenblueth, Reboul and Grass, 1940) upon the action of alternating currents on mammalian nerves showed several effects which could not be accounted for satisfactorily on the basis of the standard knowledge of the action of direct currents (d.c.). The assumption that alternating currents might exert a qualitatively specific influence was unlikely. It appeared more probable that the understanding of the effects of d.c. was incomplete or faulty.

The present study deals with the changes of electrical excitability of nerves during or after the passage of d.c.

Method. The animals used were cats, either under dial anesthesia (Ciba, 0.75 cc. per kgm. intraperitoneally) or decapitated 1 to 4 hours previously under ether. The nerve studied was mainly the motor component of the popliteal, the contractions of the muscles attached to the Achilles tendon serving as indicators of the corresponding nerve impulses.

The sciatic was cut and 6 to 10 chlorided silver-wire electrodes were applied to the popliteal. The electrodes were insulated by rubber from the neighboring tissues. The popliteal was separated from the peroneal in three stretches of about 15 mm. for the insertion of the electrodes. Care was taken in making this dissection to preserve the blood supply of the nerves as intact as possible. Observations, in which certain drugs known to affect nerves have been injected intravenously after a similar preparation, have shown that such nerves do have an adequate blood supply.

The legs were fixed by drills inserted into the tibiae. The Achilles tendon was freed and attached to the short arm of a myograph. The muscles pulled against strong rubber bands. Contractions appear in the records as upward excursions of the tracing.

In some experiments the peroneal nerve was dissected from hip to knee and excised for study in a moist chamber. The electrodes were usually thin calomel half-cells making a contact with the nerves through agar-Ringer bridges and wicks moist with Ringer. The spike potential of the

A fibers served in these cases as indicator of the nerve impulses. The potentials were recorded, after suitable amplification, from a cathode-ray oscillograph.

The d.c. was obtained from batteries, the intensity being regulated by a potentiometer. Resistances of 25,000 to 125,000 ω were usually placed in series with the nerve, in order to prevent possible spurious shifts of the stimulated points when the test shocks were delivered at either of the d.c. poles. The e.m.f. and intensity were read during the passage of the current from a voltmeter and an ammeter, respectively. The applications of d.c. lasted from 1 to 15 seconds.

In some of the classical studies on electrotonus stress was laid on the desirability of using only one direction of d.c., "ascending" away from the muscle or "descending" toward the muscle in any given experiment. That practice was not followed in this study, since it was found that the effects of a given first application could be duplicated accurately by a similar treatment following one or more applications of d.c. in the reversed direction. The only condition necessary for such accurate reduplication was a reasonable wait (1 to 5 min.) between the observations. Indeed, it was found that systematic reversal of the d.c. led to more consistent results than those obtained with repeated unidirectional treatment. In the excised nerves a slowly progressive change of effects was the rule, due probably to the absence of circulation. In the circulated nerves the early results could be reasonably reproduced even after several hours of observation as long as the intensity of the currents used was relatively moderate.

The test shocks were condenser discharges of various capacities and intensities. When these shocks were repeated regularly the frequency was controlled by a thyratron. The use of condenser discharges, instead of induction shocks, allowed the study of the polar and interpolar regions of the nerves without the establishment of additional poles for the d.c. The study of the excitability at any given point in the nerve was made with both ascending and descending test shocks. That is, while the stimulating cathode for the test shock remained fixed, the anode was made first proximal, then distal to the cathode with respect to the muscle, or *vice versa*.

Results. A. *Changes of excitability at the d.c. poles.* Increased excitability at the cathode and decreased excitability at the anode throughout the passage of even quite prolonged (several minutes) d.c. are too well known to deserve any further comment.

The decrease of excitability at the cathode found by Werigo (1883) has likewise been extensively studied. This decrease is produced either by the brief application of strong currents or by the prolonged delivery of moderate currents. In either case the intensification or prolongation of the current exaggerates further the decrease of excitability. There is,

however, another type of cathodal depression, produced by very weak currents. Intensification of the current leads in these cases to the appearance of the usual increase of excitability.

In figure 1 is illustrated a typical example of the second type of cathodal depression. As shown in the records from A to D, progressive intensification of the current causes first an increase of the depression but leads later to the appearance of increased, instead of decreased excitability. Record E shows that long interelectrodal distances are unfavorable to the appearance of depression. And record F illustrates the existence of a

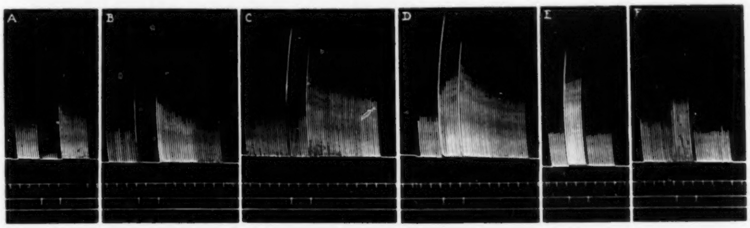


Fig. 1. The second type of cathodal depression. Cat under dial. Muscular responses to submaximal stimulation of the popliteal nerve. D.c. was applied in a descending direction, as shown by the signals. The test stimuli were applied in the ascending direction, the cathode being common to that of the d.c.—i.e., the order of the electrodes beginning centrally was: first, the d.c. anode, 6 mm. away the common cathode, and 10 mm. below that the anode for the test stimuli. A resistance of 25,000 ω was in series with the battery. The condenser discharges had a capacity of 0.01 μF and discharged through the nerve and a series resistance of 18,000 ω. The voltages of d.c. applied to the nerve were: A, 0.05; B, 0.25; C, 0.75; and D, 1.0 v.

In E the interelectrodal distance for the d.c. was increased from 6 to 12 mm. by moving the anode. The voltage of d.c. was 0.25 (cf. B).

In F the d.c. was delivered as in B. The cathode of the test stimulus was moved, however, 10 mm. peripherally to the d.c. cathode, and the anode of the test shock was moved correspondingly.

In this and the following kymograph records the time signal denotes 5-sec. intervals.

region of increased excitability outside the cathode, although the excitability at the cathode itself is depressed (B).

An increase of excitability at the anode can be easily demonstrated by several procedures. In figure 2 is illustrated a typical series of observations using condenser discharges as the test stimuli. While weak currents cause anodal depression (A), strong voltages produce increasingly augmented responses (B to D). The results are seen whether the test stimuli reach the anode of the d.c. in an ascending (B) or a descending (E) direction. While the excitability is increased at the anode (B and E) it is decreased at a point a few millimeters outside (F).

Another method used for the study of the polar changes of excitability

was the following (fig. 3). Two sources of d.c. were connected in parallel to the same pair of electrodes on the nerve. A given high resistance (10,000 to 100,000 ω) was placed in series with each of the d.c. sources.

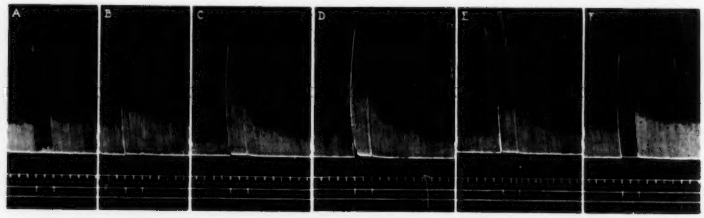
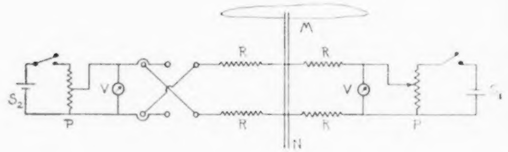


Fig. 2. Anodal increase of excitability. The procedure followed for the tests was similar to that in figure 1. The order of the electrodes from central to peripheral points of the nerve was: d.c. cathode; 19 mm. further, common electrode for the d.c. anode and the test stimulus cathode; 7 mm. further, stimulus anode. The voltages of d.c. were: A, 0.02; B, 0.05; C, 0.13; D, 0.25.

In E the anode of the test stimuli was moved to a point midway between the d.c. electrodes. The stimuli were now descending instead of ascending. Voltage, 0.05.

In F the stimulus cathode was moved to a point 7 mm. peripheral to the d.c. anode.



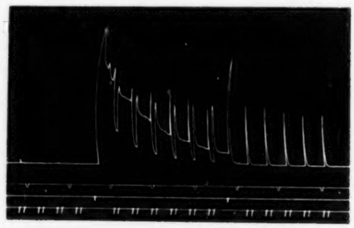


Fig. 3 Fig. 4

Fig. 3. Diagram of the arrangement for testing the electric excitability by brief d.c. pulses through the same electrodes used for continuous application of d.c. from another source. The letters have the following meaning: M, muscle; N, nerve; R, 10,000 to 100,000 ω; V, voltmeter; P, potentiometer; S, source of d.c.

Fig. 4. Increased excitability at the anode during the passage of d.c. Decapitate cat. The experimental set-up was as shown in figure 3. The voltages were measured across the resistances in series with the nerve; the drop of potential in the nerve was much less. Interelectrodal distance: 15 mm. Just threshold (1 v.) brief d.c. pulses were applied from one of the sources in the descending direction, as shown by the pairs of lower signals. The middle signal indicates the period of application of d.c. (12 v.) from the other source in the ascending direction.

The same value was used on the two circuits so that the resistance of the two loops was equal. A simple calculation shows that the drops of potential across the branch common to the two loops (that is, across the nerve) sum algebraically. In other words, the drop of potential impressed on the

nerve by one of the sources will be added to or subtracted from any drop impressed by the other source.

The experimental procedure was to apply regularly brief (0.5 to 2 sec.) descending pulses from one of the sources before, during and after the delivery of an ascending or descending prolonged (5 to 15 sec.) current from the other source. The advantages of the method are first, that, as pointed out above, the currents across the nerve are independent; second, that the responses to the make or break of the brief test pulses can be readily distinguished.

By the use of this method all the polar changes described thus far could be readily confirmed. In figure 4 is shown a typical record exhibiting increase of excitability at the anode. The preliminary test d.c. pulses caused only slight responses at the make. The prolonged ascending d.c. from the other source resulted in a sustained tetanic response. Interrupting this response are seen the now large brief tetani caused by the make of the test pulses. Such make is equivalent to a sudden weakening of the d.c. applied, since the two currents flowed in opposite directions. The drop in the sustained tetanus during the closure of the test is due to this relative weakening. The opening of the tests is equivalent to a sudden intensification of the continuous current, and results in turn in a strong tetanic response. A post-anodal increase of excitability is shown by the large responses to the test stimuli after the prolonged d.c. was interrupted.

An independence of the polar effects was indicated by the fact that when d.c. of a certain intensity increased the excitability at one pole it could cause either an increase or a decrease at the other pole. This independence is illustrated in figure 5.

In some of the experiments the polar changes of excitability were studied, using electrodes shielded by glass (Sherrington electrodes) for the application of d.c. The purpose of these observations was to isolate the tested region of the nerve from surrounding tissues. Although higher voltages were necessary for the appearance of some of the changes described above, they could all be confirmed. The relatively greater difficulty found for the demonstration of anodal enhancement or cathodal depression of the type illustrated in figure 1 is attributed both to impairment of the blood supply of the nerve and to the proximity of the region tested to the cut end.

The importance of the blood supply was seen in controls in which this supply was abolished, leaving the nerve *in situ* and placing the electrodes as usual. In such nerves the effects described above were difficult to obtain or did not appear at all. The importance of testing regions of the nerve at some distance from the cut end was readily demonstrated by applying similar tests first near and then far from the cut. In the first case only Pflüger's classical effects could be shown.

B. *Juxtapolar changes.* A quantitative description is given below

(section G) of the changes of excitability throughout all the regions of the nerve, polar, interpolar and extrapolar, produced by the applications of d.c. The purpose of this section is to emphasize that the effects at the poles may be quite different in sign and magnitude from the changes in regions of the nerve distant only a few millimeters away. These differences are especially noticeable when the currents applied lead to an increase of excitability at the anode or a decrease at the cathode (see figs. 1 and 2).

C. *Distant extrapolar changes.* Wedensky (1920) reported that when the tests of excitability were carried out far enough away from the d.c. poles the sign of the change was reversed. Thus, if an ascending current resulted in a decreased excitability of the regions of the nerve within 2 or

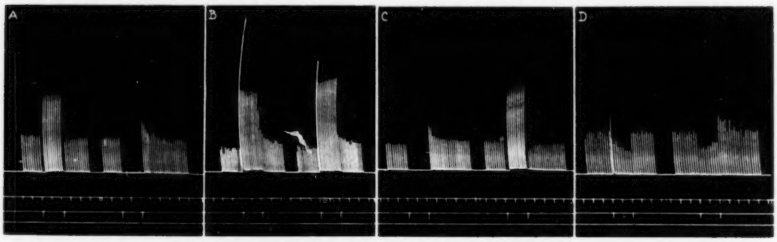


Fig. 5. Independence of the polar effects. All the tests were made using the pole of the d.c. proximal to the muscle as the cathode for ascending test shocks. The records are arranged in pairs, showing the effects at the same point, first of an ascending, then of a descending d.c.

A. Anodal increase and cathodal decrease. Voltage of d.c. 0.05. Interelectrodal distance: 6 mm.

B. Anodal and cathodal increases. Voltage: 0.1. Interelectrodal distance 6 mm.

C. Anodal decrease and cathodal increase. Voltage: 0.1. Interelectrodal distance 15 mm.

D. Anodal and cathodal decreases. Voltage: 0.1. Interelectrodal distance: 12 mm. The test capacity was smaller (0.001 μF) than the one used for the other tests (0.01 μF).

3 cm. from the anode, the excitability of regions distant 4 to 6 cm. from that pole was increased. Conversely, the cathodal extrapolar segment showed first an increase, then a decrease of excitability as the points tested were further from the pole. Wedensky spoke of the regions of the nerve where this reversal was found as the peri-electrotonic zone.

Wedensky's report was easily confirmed. The reversal of the excitability changes in the extrapolar regions was seen even with weak currents. For a given distance between the d.c. electrodes the stronger the current, the closer to the pole was the point at which reversal took place.

D. *The measurement of the changes of excitability.* A change of excitability could be due to a modification of either the intensity or the time parameter or of both parameters of the strength-duration curves of the

nerve fibers. The qualitative determinations illustrated in figures 1, 2 and 5 merely indicate that the nerve as a whole has become hyper- or hypoexcitable in a certain region, since more or less fibers, respectively, are activated by the invariant test stimulus.

In order to gain a more accurate insight of the changes produced, voltage-capacity curves were constructed as follows. The end-point was a response of constant small magnitude (usually about 20 per cent of the maximal twitch). This insured the test of a constant number of fibers. The d.c. and the test-stimulating electrodes were maintained in a fixed position. The normal voltage-capacity curve (without d.c.) was first determined in the usual manner. The normal intensity value for a given capacity was then re-ascertained. A certain fixed voltage of d.c. was then applied and maintained until the voltage of the test stimulus for the constant response was determined (3 to 5 sec.). The d.c. was discontinued. One to 5 minutes later the normal value was checked, the d.c. was re-applied but in the reversed polarity, and the new threshold was determined as before. The procedure was then repeated with another capacity until the whole range of the curves had been explored. The repeated test of the normal values first obtained insured that no progressive change was present which would vitiate the results. The determinations were made first with decreasing and then with interpolated increasing capacities. The three curves (normal, anodal and cathodal) were plotted in double logarithmic scales and analyzed for the values of the voltage (r) and time (k) parameters, according to Hill's (1936*b*) method.

The results were as follows. The outstanding change produced by d.c. was in the voltage parameter (rheobase), but the time parameter was invariably likewise affected. As a rule, a decrease of rheobase was attended by an increase of k and *vice versa* (fig. 6A). An increase of the time parameter denotes decreased excitability. A decrease of rheobase leads, on the contrary, to greater excitability. Since both changes usually took place simultaneously, it is clear that the net modification of excitability depended on which of the two changes was more prominent. As already stated, the rule was for the change in rheobase to determine the final reactivity for all durations (fig. 6A). It was possible, however, to obtain conditions in which the electrotonic curve crossed the normal curve (fig. 6B). In such cases, if the test was made with a brief shock the nerve appeared hypoexcitable during the passage of d.c., whereas it appeared hyperexcitable if the test shock was of relatively long duration.

Since the changes of excitability produced by d.c. involve both the voltage and the time parameters it is not possible to measure such changes in a simple direct manner. The predominant change, however, is that in the rheobase. It was decided, therefore, that an approximate measurement could be reached by determining the voltage threshold for a response

of fixed magnitude with a relatively large capacity. The ratio of the normal value of this threshold to the value obtained during an experimental procedure multiplied by 100 expresses the percentile modification of excitability.

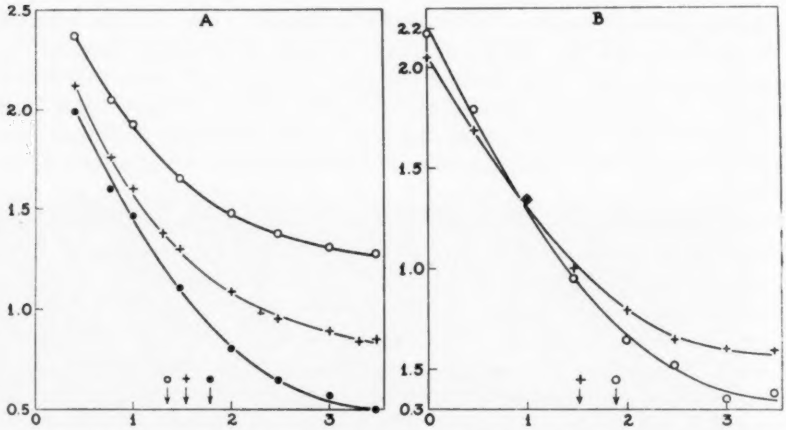


Fig. 6. Voltage-capacity curves during the passage of d.c. The curves are plotted in logarithmic scales. Abscissae: $\log_{10}$ capacity; the unit is 0.001 μF. Ordinates: $\log_{10}$ voltage; unit, 0.1 v. The scale for the ordinates is twice that of the abscissae. The arrows indicate the value of Hill's constant k.

A. The interelectrodal distance for the d.c. was 16 mm. The cathode of the test stimuli was 6 mm. below the peripheral d.c. pole. Resistances of 9,000 ω and 1,800 ω were placed in series and in shunt, respectively, in the condenser circuit. Crosses: normal curve, without d.c.; some of the points were taken before, some after the applications of d.c. for the construction of the other curves. Circles: curve obtained during applications of ascending d.c. (0.5 v.; anodal polarization). Dots: curve obtained during applications of descending d.c. (same voltage; cathodal polarization).

B. Interelectrodal distance for the d.c.; 10 mm. Test cathode at the peripheral pole of the d.c. Resistances of 10,000 ω were in series and in shunt with the nerve in the condenser circuit. Crosses: normal curve. Circles: during applications of ascending d.c. (0.5 v.; anodal polarization). The curve obtained during cathodal polarization is omitted in order not to obscure the crossing of the normal and anodal curves.

E. *The influence of voltage.* Changes in the intensity of the d.c. applied resulted not only in quantitative variations of the effects observed, but could result also, as already pointed out (figs. 1 and 2), in a reversal of the sign of a given effect. Using the method just described for the measurement of the electrical excitability, curves were traced correlating the sign and magnitude of the changes with the voltage of the currents applied.

No uniform description of these curves can be given because the results

varied with the interelectrodal distance for the d.c. and the region of the nerve tested. The extreme changes of excitability found were 350 and 20 per cent of the normal. The curves often crossed the line of normal excitability. Thus, low voltages could produce a decrease and higher voltages an increase of excitability at the same point of the nerve and with a constant polarity of d.c. (see figs. 1 and 2).

Qualitatively the following statements summarize the influence of voltage on the variations of excitability. Weak currents favor the appearance of a decrease at the anode and cathode. Stronger currents promote increased excitability at both the anode and the cathode. With even stronger currents the increase at the anode grows, whereas the increase at the cathode declines or is replaced by a decrease. All the intrapolar and extrapolar changes become more prominent with increasing voltage.

F. *The influence of the interelectrodal distance.* The distance between the d.c. electrodes was of importance in the results obtained. With equal voltage drop across the electrodes or equal intensity of current flowing through the nerve, variations in the interelectrodal distance resulted not only in quantitative differences of effects (Pflüger, 1859) but also could result in a reversal of sign of the change of excitability elicited at a given point.

The most marked effect seen in this respect was on the appearance of an increase of excitability at the anode. Thus, 2 to 8 times stronger voltages were necessary to elicit an anodal increase if the interelectrodal distance was doubled, for instance, from 6 to 12 mm., by moving the cathode without disturbing the anode. A relatively short span of the d.c. poles was also found to favor the appearance of cathodal depression.

Not only the distance between the d.c. poles, but also the length of nerve included between the two electrodes used for the delivery of the test shocks was of importance for the results recorded. Thus, anodal enhancement could be obtained with weaker d.c. if that length was short than when the stimulating electrodes were widely separated. Proper controls showed that the influence of the length in question was more significant than that corresponding to the direction, similar or opposite, of the test current with respect to the d.c.

G. *The distribution of effects along the nerve.* The mapping of the changes of excitability at different regions of the nerve produced by a given application of d.c. was effected as follows. Referring to the 10 electrodes on the peroneal nerve by numbers, *1* to *10*, two of them were selected for the applications of d.c. with constant voltage, e.g., *3* and *5*. A given test capacity was then discharged in the descending direction from *1* to *2*, and the voltage necessary for a fixed response was determined. This value, obtained without d.c., gave the normal threshold for that position of the test. The d.c. was then applied with the ascending polarity and the thresh-

hold was redetermined within 3 to 5 sec. After a pause, the normal value was checked, d.c. was applied in the reversed direction, and the new threshold was determined. The changes of excitability at that point could then be calculated as explained in section D. The test was then moved systematically to the next two electrodes on the nerve and the procedure was repeated. The measurements were carried out until electrodes *9* and *10* were reached and then the series was repeated with ascending tests in the reversed direction: *10, 9; 9, 8;* etc.

The values obtained were attributed to the point of the nerve where the cathode of the test was placed. There were, therefore, two values for each of the electrodes *2* to *9*, and one for the electrodes *1* and *10*, for each of the directions of the d.c. The curves were plotted with the distances between the points tested and the d.c. poles as abscissae, and the corresponding changes of excitability as ordinates.

In order to make the changes seen with one polarity of d.c. comparable with those obtained with the reversed polarity, the plot was first made of all the values obtained with the ascending current. The values for the descending current were then plotted on the same graph, using the points already marked for the anode and cathode as references for the abscissae. In other words, whether the observations were made with an ascending or a descending current, the changes at the cathode, or those at a given distance from the cathode in the cathodal extrapolar region, etc., were plotted at the corresponding point on the graph. With this procedure at several of the points in the abscissae there were as many as four values for the ordinates.

The results were quite consistent in any series of determinations. Occasionally, some discrepancies were seen when comparing the values obtained with the ascending or descending d.c., or with the ascending or descending test stimulus, for a given point on the nerve. These discrepancies were more frequent for the d.c. poles than for any other region of the nerve. But even when such discrepancies were present a repetition of any of the determinations at the end of the series agreed with the values obtained previously.

As shown in the preceding sections, at least four independent variables are involved in the changes of excitability produced by d.c. at a given point of the nerve: d.c. interelectrodal distance, d.c. voltage, distance of the point from the d.c. poles, and duration (capacity) of the test stimulus. Even if the last factor is dismissed from consideration for the reasons given in section D, only a family of surfaces could give a complete graphical description of those changes. Rather than attempting to project such a family of surfaces into a two-dimensional drawing, some experimental curves are illustrated in figure 7.

Curve A is typical for a moderate interelectrodal distance and intensity

of d.c. The curve is fairly similar to Pflüger's (1859) classical law, but a slight extrapolar reversal is clear. With slightly shorter interelectrodal distances the maximum and minimum of the excitability changes were displaced to the extrapolar regions. Curve B shows the presence of anodal enhancement. In many instances the increase at the anode was only relative, that is, the excitability was depressed, but less that in neighboring inter- and extrapolar regions. In curve C both anodal enhancement and cathodal depression are present. Cathodal depression

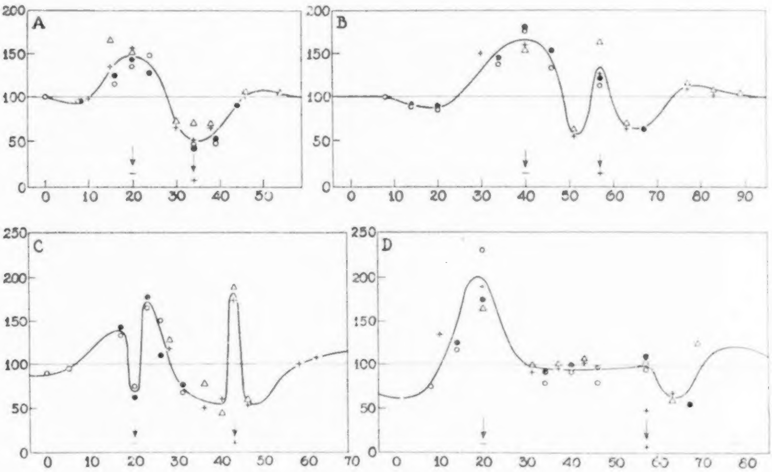


Fig. 7. Different types of curves describing the distribution of excitability changes in different regions of a nerve upon applications of d.c. Ordinates: per cent excitability (referred to the normal). Abscissae: distances in millimeters along the nerve. The arrows and signs indicate the position of the d.c. electrodes on the nerve. Crosses and triangles: points determined for ascending d.c. with ascending and descending stimuli, respectively. Circles and dots: values for descending d.c. with ascending and descending stimuli, respectively. Further explanation of the method in text. The voltages of d.c. were 0.3, 0.5, 0.5 and 0.8 for A to D, respectively.

was in turn sometimes only relative—i.e., less increase than in neighboring points. Curve D is typical of long interelectrodal distances, in showing practically normal excitability in most of the interpolar region.

H. *The lack of polarity in motor fibers.* By polarity in a nerve fiber is meant any systematic change along the longitudinal axis such that the more central region of the fiber would exhibit properties differing quantitatively from those of more peripheral regions. An absence of polarity with regard to electrical excitability was shown by two sets of observations. First, the threshold of the nerves tested with either ascending or descending

condenser discharges of a fixed capacity (0.001 to 1μF) varied only in a random and slight degree for points of the nerve from 3 to 8 cm. below the cut central end, provided the interelectrodal distance was approximately constant. The random variations can be explained by slight differences in the contacts made by the corresponding electrodes with the nerve. Second, the determinations of changes of excitability made with ascending currents agreed quantitatively, as a rule, with those made with descending currents (fig. 7).

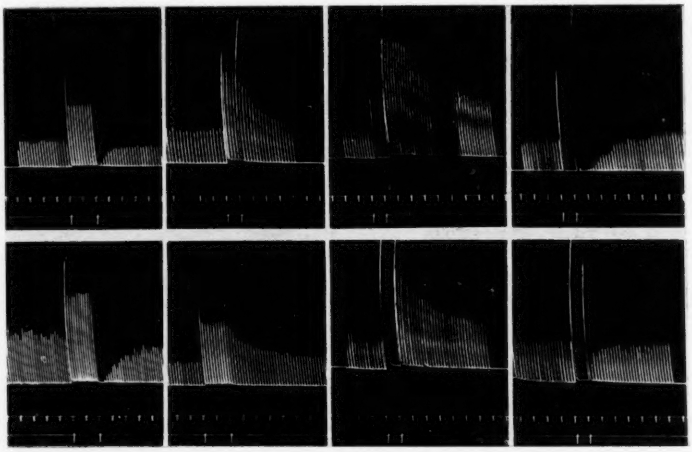


Fig. 8. Independence of the after-effects of d.c. from the changes produced during the passage of the current. All the tests were made with the cathode of the test stimulus common to the peripheral d.c. pole. The stimuli were ascending, that is, the anode was further peripherally. The upper row of records is for ascending d.c.—i.e., tests of the anodally polarized point. The lower row is for descending currents—i.e., tests of cathodally polarized point. None of the currents used was strong enough to produce a block of nerve impulses.

It is obvious that if axons are not polarized, but uniform, the distinction between ascending and descending currents is unimportant except when blocking effects are developed. For this reason the direction of the current in the nerve was not qualified in sections A to F.

I. *The after-effects of d.c.* The results described thus far have dealt with effects encountered during the passage of d.c. Because there is no reason to assume that the after-effects of a given application of current differ fundamentally from the changes encountered during the application, no quantitative study of these after-effects was attempted. Some interesting features of the numerous qualitative observations made are the following.

The after-effects at a given point may or may not have the same sign as the change produced during the passage of d.c. (fig. 8). In some instances, especially with relatively strong voltages of d.c., first an increase and later a decrease of excitability was observed, or *vice versa*.

Post-cathodal depression is apt to occur after weak currents, especially if the interelectrodal distance is long. Strong voltages, on the other hand, favor the appearance of a post-cathodal increase of excitability (Pflüger, 1859). Post-anodal depression is likewise more prominent after strong than after weak currents. With a constant interelectrodal distance greater voltages were usually necessary to convert the post-anodal increase of weak currents into a post-anodal depression than those sufficient for the transition from a post-cathodal depression to an increase.

J. *Observations on excised nerves.* As mentioned under Method, the indicator of nerve activation was in these cases the spike potential of the A fibers. The d.c. was delivered through non-polarizable calomel electrodes. The results differed from those in the circulated nerves as follows.

Anodal enhancement of excitability was only seen with quite short (3 to 6 mm.) interelectrodal distances. A further important precaution for the appearance of this effect was to test a region of the nerve well away (at least 3 cm.) from the cut ends. With this precaution very weak voltages led to classical depression at the anode during the passage of d.c. Slight intensification of the current (0.02 to 0.04 v. across nerve) resulted in anodal enhancement. Further intensification (e.g., 0.1 v.) caused again a depression. Figure 9 illustrates typical observations.

Reversal of the extrapolar cathodal effects—i.e., transition from increase to decrease of excitability—was readily seen. The similar reversal in the anodal extrapolar region (p. 62) was not seen clearly in any instance.

Discussion. A comparison of the present results with those obtained by Pflüger (1859) shows that Pflüger's observations were accurate but incomplete. His interpolations and extrapolations were, therefore, inadequate. His observations would fit the curves in figure 7, yet some of these curves differ considerably from the classical laws reproduced in all text-books. Instead of the one "indifferent point" separating the anelectrotonic, depressed zone, from the catelectrotonic, enhanced region of the nerve, there may be as many as 7 "indifferent points" during an application of d.c. (fig. 7C).

Cathodal depression (Werigo, 1883) and anodal increase of excitability (for references see Mares, 1913; Thörner, 1923; and Ebbecke, 1933) have been frequently reported. But the appearance of these effects has been usually considered indicative of abnormality of the nerves. Blair and Erlanger (1936) describe a cathodal depression in normal nerves treated with weak currents, but this depression is only relative; instead of an absolute decrease of excitability it appears in their observations as a reduction of the original increase produced by the current.

The cathodal depression and anodal increase shown in figures 1, 2, 4, 5 and 8 cannot be attributed to abnormal conditions. The effects appeared with weak currents and the nerves were kept as fresh and intact as possible. Indeed, as emphasized in sections A and J, the phenomena were less clear or could not be obtained in abnormal nerves, nerves deprived of their circulation or excised.

Evidence of anodal enhancement can be shown by a procedure different from those employed here (Rosenblueth, 1941a). Impulses traveling over some of the fibers in a nerve may stimulate adjacent fibers at the region of the anode of an applied d.c. The phenomenon is only seen in fresh nerves. Again, an increase of excitability at the anode during the passage of d.c. sufficient to result in excitation of the nerve is best seen in normal nerves (Rosenblueth, 1941b).

In an effort to bring together all the effects of d.c. upon nerve in a relatively simple schema, Ebbecke (1933) suggested that under the influence of increasingly stronger cathodal polarization a nerve's excitability is first increased to an optimum but later depressed until total failure to conduct takes place (see Wedensky, 1903). The anodal influence would be opposite in character to the cathodal effect. Thus, depending on the state of the nerve at the time of the test either cathodal or anodal polarization could increase or decrease excitability by causing the nerve to approach or depart from the condition of optimal excitability.

There are many observations not explained by this simplified schema. According to it, whenever weak cathodal or anodal polarization causes a decrease of excitability, intensification of the current should lead to greater depression, because the state of the nerve would be moved further away from the condition of optimal excitability. Intensification may lead, however, from depression to enhancement (figs. 1 and 2). Furthermore, according to the schema, weak currents should invariably result in opposite effects at the anode and cathode. This corollary is not supported by the observations in figures 5 and 9.

The following factors were found to influence the effects of d.c.: *a*, distance between the d.c. electrodes (figs. 1 and 2, section F); *b*, voltage (figs. 1, 2 and 9; section E); *c*, distance of points tested to the d.c. poles (figs. 1, 2 and 7; section G); *d*, duration of the test shocks (fig. 6; section D); *e*, interelectrodal distance for the test stimuli (section F). Although the last two variables are less important than the others for certain purposes, it is clear that no simple schema will do justice to the facts. Ebbecke's hypothesis considers only the influence of voltage. It is a 2-dimensional representation, whereas at least a 4-dimensional description is required by the data.

The term "accommodation" was introduced by Nernst (1908) to describe a rise in the threshold of nerve when a steady potential is applied

for some time. It is obvious from this definition that the concept of accommodation and the study of electrotonic changes of excitability are intimately related.

In developing his comprehensive theory of electrical excitability of nerve, Hill (1936a) dismissed electrotonic phenomena as secondary, on the basis of observations showing a decrease or even an inversion of the "usual" electrotonic effects. The fact that electrotonic changes are contingent upon the experimental procedure is not an adequate argument for considering them as secondary in the problem of excitation. Operationally the modifications of excitability produced by d.c. are inseparable from some of the facts recognized by Hill as of primary importance, e.g., the facts which lead to the concept of accommodation.

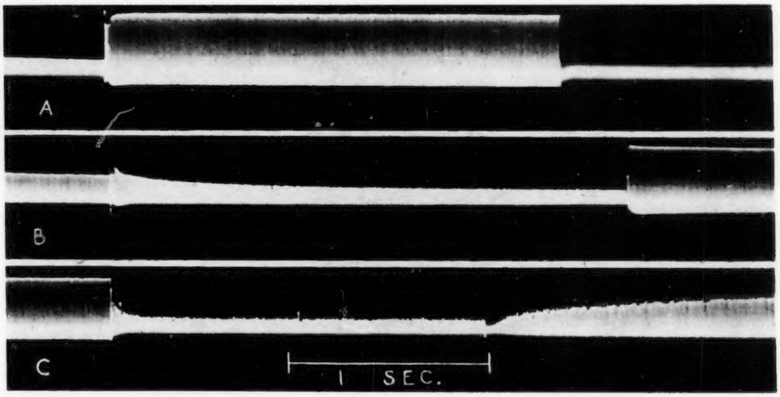


Fig. 9. Increased excitability at the anode during the passage of d.c. Excised peroneal nerve. Monophasic records of the spike potentials of A fibers. Submaximal test stimuli applied continuously at the rate of 120 per sec. The sudden changes in the records correspond to the closure and opening of the d.c. The distance between the d.c. electrodes was 5 mm. The anode of the d.c. coincided with the cathode of the test stimuli. Voltages of d.c.: A, 0.04; B, 0.1; C, 0.2.

Hill defines "normal accommodation" as a change in threshold such that when a nerve is at equilibrium with a given current the additional current required to stimulate is the same as it was originally, before any application of d.c. This equilibrium would be approximately attained within a very brief period after the application of d.c. As a simplification that definition may be useful. But the term "normal" is unsatisfactory. It is only very exceptionally, if ever, that this type of accommodation is seen experimentally. In optimum normal conditions, that is, in unanesthetized, circulated nerves *in situ*, applications of d.c. result in changes of excitability which persist for minutes not only at the poles but also at

points up to 6 to 8 cm. away from the poles. Such applications may cause prolonged tetanic discharges if the voltage is adequate (Rosenblueth, 1941*b*). A general definition of accommodation should include all the electrotonic changes, not only some selected instances. With the data available there is no reason for separating the electrotonic phenomena from the phenomena of excitation.

The classical approach to the problem of the action of electricity upon nerve is to assume that the changes of excitability produced are direct consequences of the changes of polarization caused by the current: depolarization at the cathode and increased polarization at the anode. This assumption is inadequate to cope with the complex effects encountered. It appears more likely that the action of the current is not direct, but that intermediate physico-chemical steps may play a rôle. Such hypothetical intermediate processes would not necessarily be opposite at the anode and cathode.

The data (figs. 1, 2, 4 and 9) suggest that both the cathodal and the anodal influences include a factor for increasing and another for decreasing the excitability of nerve. The preponderance of one or the other of the two factors in a given region of the nerve is determined by the experimental conditions. An independence of the several influences is supported by the lack of uniformity seen when comparing the anodal or cathodal effects (fig. 5) or after-effects (fig. 8). An interaction of the anodal and cathodal actions is seen, on the other hand, in the asymmetrical distribution of the changes at different points in the interpolar region of the nerve (fig. 7).

SUMMARY

The changes of electrical excitability produced by application of direct current (d.c.) were studied in cat's myelinated nerves, either circulated or excised.

There may be an increase or a decrease of excitability at the anode or the cathode during or after the passage of d.c. (figs. 1, 2, 4, 5, 8 and 9).

The following factors influence the results obtained: *a*, distance between the d.c. electrodes (figs. 1 and 2, section F); *b*, voltage of the d.c. (figs. 1, 2 and 9; section E); *c*, distance of the points tested to the d.c. poles (figs. 1, 2 and 7; section G); *d*, duration of the test shocks (fig. 6; section D); *e*, interelectrodal distance for the test stimuli (section F).

The changes of excitability are due to modifications of both the voltage and the time parameters of the voltage-capacity curves (fig. 6).

The distribution of effects along the nerves may be more complicated than has been assumed hitherto (fig. 7).

For a given application of d.c. the change at the anode does not permit predicting the effect at the cathode, and *vice versa* (fig. 5). Similarly, the

after-effects at either pole cannot be predicted from the effects seen during the application (fig. 8).

The results are discussed in relation to the problem of the action of d.c. upon nerve and in relation to theories of electrical excitability.

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THE RESPIRATION OF ISOLATED LIVER AND KIDNEY TISSUES FROM ADRENALECTOMIZED RATS

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It is generally recognized that the energy necessary for the maintenance of the normal activities of liver and kidney tissues, and presumably of all tissues in the animal body, arises from oxidation-reduction processes chiefly. It is known now that, as a rule, electrons are transferred through a long chain of dehydrogenases through reversible redox systems from the oxidizable substance to molecular oxygen (1). In animal tissues the rate of oxidations is controlled not only by conditions which ordinarily affect the rate and equilibrium point of chemical reactions, but also by the influence of hormones. The specific nature of the latter factor is not very well understood. Damage of the enzyme systems concerned in oxidation must be an important factor in the development of many abnormal conditions of which adrenal insufficiency may be one.

Many abnormal conditions have been found to affect oxidations in the cells. Barron (2) has shown that Rous chicken sarcoma and also infectious myxoma in the rabbit do not oxidize succinic acid. It was further found that many tissues under the influence of filterable virus diseases oxidize it more slowly than normal tissues. Liver slices from rabbits suffering from diphtheria toxemia show a diminution in the power to oxidize lactic and pyruvic acids (3). The marked neural and muscular disturbances that are common to adrenal insufficiency in all species so far studied, together with the depression in basal heat production (the BMR may fall as much as 25 per cent below normal as crisis develops) which occurs at least in rats (4), suggest very strongly that oxidative systems may be adversely affected in the absence of the adrenal cortical hormones. A recent report by Crismon and Field (5) suggest that this may be particularly true of kidney tissue.

The results here reported show that adrenal insufficiency in rats results in a significant depression of the respiration of liver and kidney slices developing about a week after the operation, associated with a considerable diminution in the rate of oxidation of pyruvate and succinate by liver tissue.

METHODS. *Condition of the animals.* Young male rats weighing from

85 to 100 grams were selected for the tests. Litter mate controls were used where possible but the control group includes some animals who have no litter mates in the experimental group. The same is true of the experimental group. Bilateral adrenalectomy was carried out at a single operation. In our hands the average survival time of rats after adrenalectomy is 11 days. Consequently the measurements of oxygen consumption were made on the 7th to 10th day following the operation. The tested animals usually showed obvious signs of insufficiency.

The data on the experimental rats are taken only from animals which showed no accessory bodies at autopsy and a progressive loss in weight (as compared to controls) after the fourth day after operation. Sham operations were carried out on some controls. After recovery there was no appreciable difference from the other controls so this procedure was not generally followed.

In general the adrenalectomized animals received no hormonal or salt treatment. For some tests, however, two variations were made: *a*, some of the adrenalectomized rats were given Rubin-Krick's solution (6) as drinking water, and *b*, some of the control animals were fed the same quantity of food which their adrenalectomized litter mates consumed voluntarily.

Determination of Q_{O_2}. In general the same procedure was followed as described in a previous paper (7). The tissue slices were sectioned with a double blade cutter (8) adjusted to give a section thickness of less than 0.3 mm. Such a thin section is particularly necessary in determining accurately the high oxygen consumptions resulting from succinate oxidation. The kidney slices were sections taken from the central part of the kidney passing through cortex and medulla.

Sodium pyruvate, sodium succinate, and dextrose in concentrations of 0.04 M. previously buffered at pH 7.3 were added to the Ringer's solution at the beginning of those experiments designed to test the ability of the tissues to oxidize these metabolites. Oxygen consumption and CO_2 production (in tests where the R.Q. was measured) were determined in differential microvolumeters. The Q_{O_2} as usual is expressed in cubic millimeters per milligram dry weight per hour and is given as a positive figure. The suspension medium was Ringer's solution buffered at pH 7.3 with 0.02 M. phosphate buffer.

All tests were made with the animals under basal conditions.

RESULTS. *Liver.* Table 1 presents all the data obtained on the respiration of liver slices from normal and adrenalectomized rats. The "fed normals" were placed on the ordinary stock diet and allowed to eat all that they desired. The "partially-starved normals" were fed the same quantity of food that adrenalectomized litter mates ate voluntarily. Insufficiency is characterized by anorexia so the litter mate control was

starved to some extent. The "adrenalectomized" group received all the food that they desired and tap water for drinking. The "salt-treated adrenalectomized" received Rubin-Krick's salt solution (6) as drinking water from the time of operation until the respiration test. The tests were made from 7 to 10 days after the operation. The exact time depended upon the condition in which the animal appeared to be; that is, how close it was to collapse.

Adrenalectomy decreases the rate of oxygen consumption of liver slices from a mean value of 8.68 c.mm./mgm./hr. for the normals to 5.86

TABLE 1

The oxygen consumption of liver tissue from normal and adrenalectomized rats under basal conditions

The effect of different metabolites is also given

	TIME AFTER DEATH	CONTROL	SODIUM PYRUVATE, 0.04 M	SODIUM SUCCINATE, 0.04 M	DEXTROSE, 0.04 M
Fed normals (20 animals)					
		Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}
Mean	1st hour	8.68 ±0.15*	14.01 ±0.53	17.27 ±0.53	9.48 ±0.21
	2nd hour	8.11 ±0.12	13.7 ±0.47	16.93 ±0.42	9.02 ±0.19
	R.Q. =	0.61 ±0.01			
Partially-starved normals (3 animals)					
Mean	1st hour	7.95	13.0	15.45	8.65
Adrenalectomized (20 animals)					
Mean	1st hour	5.86 ±0.12	7.13 ±0.17	9.88 ±0.34	6.38 ±0.10
	2nd hour	5.47 ±0.11	6.88 ±0.14	9.45 ±0.29	6.16 ±0.12
	R.Q. =	0.64 ±0.02			
Salt-treated adrenalectomized (5 animals)					
Mean	1st hour	7.35	11.1	13.3	8.82

* Standard error of the mean.

for the adrenalectomized group. It will also be noted that the Q_{O_2} decreases in the second hour of the test to about the same extent in both normals and adrenalectomized groups. In the presence of substrate the decrease with time is not so marked. Partial starvation depresses the Q_{O_2} but to a smaller extent than does adrenalectomy. The liver slices from the salt-treated group have a rate of oxygen consumption approaching that of the partially-starved, unoperated animals.

As shown in table 1 pyruvate, succinate and dextrose (0.04 M) increase the oxygen consumption of liver tissue of well-fed and also of the partially-

starved normal rats. The Q_{O_2} of liver of the adrenalectomized group is stimulated also but as table 2 brings out, the percentage increase resulting from the addition of these substances is not so great as is the case in the unoperated normal group. Dextrose has the least effect, raising the Q_{O_2} in both normals and adrenalectomized rats about 10 per cent. Succinate shows the greatest effect producing a 100 per cent increase in normal rats but having a somewhat smaller influence on adrenalectomized animals. Very little of this decrease in rate of oxidation of succinate that is found in the operated rats can be attributed to starvation since, as table 1 indicates, the influence of succinate on the livers of partially-starved animals differs very little from that exerted on well-fed normals. Rosenthal (9) found that succinate oxidation was relatively independent of the nutritive condition of the rat. The effect of adrenalectomy suggests that in the absence of the cortical hormone the enzyme systems necessary for the

TABLE 2

The percentage change in oxygen consumption of the liver tissue resulting from the addition of metabolites as taken from data in table 1

TIME OF TEST	CONTROL	SODIUM PYRUVATE	SODIUM SUCCINATE	DEXTROSE
Normals				
	per cent	*per cent*	*per cent*	*per cent*
1st hour	0	+61.4	+98.9	+9.2
2nd hour	0	+68.9	+108.7	+11.2
Adrenalectomized				
1st hour	0	+21.6	+68.6	+8.87
2nd hour	0	+25.7	+72.7	+12.6

oxidation of succinic acid are depressed slightly. Von Szent-Györgyi (10) has shown that succinic acid and its oxidase are important components of the carbohydrate oxidation systems.

The rate of pyruvate oxidation by liver tissue is markedly decreased after adrenalectomy falling from 61.4 per cent stimulation in the normal group to 21.6 per cent in the animals having insufficiency of adrenal hormones. The oxidation of pyruvate in the latter group still proceeds actively but at a considerably slower rate than in normals. Pyruvate increases the respiration of liver of starved rats about as much as it does that of well-fed ones. In no case was there a depression of respiration as Rosenthal (9) found with pyruvate after long periods of starvation. Pyruvate is an important intermediary metabolite in carbohydrate and protein oxidation and a disturbance in its oxidation might result in an altered metabolism. However, the respiratory quotient does not differ

significantly in the normal and adrenalectomized groups (table 1). Apparently the residual pyruvate oxidation is adequate to maintain the same type of metabolism as is found in the normal rats. Salt-treated animals show values reverting toward the normal.

Kidney. Table 3 gives the results found for kidney tissue. Kidney tissue taken from rats suffering from adrenal insufficiency has a lower Q_{O_2} than the normal. The decrease shown here (18.66 c. mm./mgm./hr. to 13.73) agrees fairly well with the data recently reported by Crismon and Field for rats on the 7th and 8th days after operation.

TABLE 3

The oxygen consumption of kidney tissue from normal and adrenalectomized rats under basal conditions

NORMALS		ADRENALECTOMIZED	
Animal	Q_{O_2}	Animal	Q_{O_2}
STK1	18.5	STK1a	14.5
STK2	20.5	STK2a	12.7
STK2b	19.65	STK2c	15.3
STK3	17.55	STK3a	13.8
STK4	17.83	STK4a	10.73
STK4b	18.2	STK4c	13.5
STK5	19.3	STK5a	14.75
STK6	18.42	STK7	16.0
STK8	19.73	STK8a	13.22
STK9	17.34	STK9a	12.8
Mean............	18.66 ±0.31*		13.73 ±0.45

* Standard error of the mean.

Discussion. It has been shown that the oxygen consumption of liver and kidney slices is depressed in the later stages of adrenal insufficiency. Since summated tissue respiration amounts to at least 66 per cent of the basal oxygen consumption of the whole animal (11) the decrease in kidney and liver respiration might account for a significant part of the fall in basal metabolism which occurs in adrenalectomized rats. The fact that brain (7, 5) and skeletal muscle (5) show little change in oxygen consumption even in the late stages of adrenal insufficiency (the same has been found in this laboratory to be true of diaphragm muscle), suggests that a change in functional metabolism is also an important factor in the depression of the metabolic rate. It seems surprising that tissues such as brain and skeletal muscle which show such marked changes in functional activity after adrenalectomy should manifest such stable oxidative systems.

Substrate concentration may be a factor in the depression of Q_{O_2} of liver in adrenalectomized rats. In table 1 it will be noticed that the liver

Q_{O_2} is lower in partially-starved rats than in well-fed normals and it is restored by adding pyruvate or succinate to the medium. The adrenalectomized rat suffers considerable anorexia and the resulting subnutritional state may act to lower the rate of oxygen consumption of the tissue.

Marked depression of the rate of oxygen consumption in adrenalectomized rats does not develop until about the sixth day after operation, or possibly the fifth (5). This, coupled with the finding that cortical hormone has no stimulating effect on the tissue respiration when added to the medium (the effect is one of depression actually (7)) raises the possibility that the results reported in this paper may be indirect consequences of cortical hormone deficiency and not direct ones. The action may be on the liver and kidney through the thyroid-pituitary complex.

The pyruvate oxidation system in animal tissues is composed of a dehydrogenase, diphosphothiamine, alloxazine and cytochrome-oxidase. The fact that after adrenalectomy in rats there is still an active pyruvate oxidation indicates that there is no severe disturbance of the enzyme chain, that is, that the chain is still intact, but some one or more of the various components may be weak in concentration or partially blocked. It may be possible to substitute ferricyanide as the oxidizing agent in place of cytochrome-oxidase (12) and thus determine whether that component has been affected. At present it is impossible even to suggest where the deficiency may be.

The rate of oxidation of succinic acid by liver is less depressed than pyruvate oxidation by adrenalectomy. If, as Rosenthal (9) suggests, the rate of succinate oxidation may be used as a measure of the minimum acitivity of the cytochrome-oxidase system, the implication is that cytochrome-oxidase is depressed to some extent by adrenalectomy. The greater depression of the pyruvate oxidation indicates that some one or more of the other components in addition to cytochrome-oxidase are depressed.

Lyman and Barron (13) have found that the oxidation of pyruvate, succinate, and the synthesis of carbohydrate from pyruvate by kidney tissue is appreciably decreased when the kidney is damaged by diethylene glycol. Evidence is given that the drug acts particularly on activating proteins (dehydrogenases). Since renal malfunction is so marked in the secondary changes resulting from adrenal insufficiency it seems desirable to determine the nature of the enzyme deficiency causing the respiratory depression shown in table 3.

SUMMARY

Adrenalectomy results in a decrease in the Q_{O_2} of liver slices from a mean value of 8.68 found in well-fed normal rats to a mean value of 5.86 and a decrease in Q_{O_2} of kidney slices from a mean of 18.66 to 13.75. The

decrease in respiration is shown 7 to 10 days after the operation. The type of substances metabolized is unaffected by adrenalectomy since the respiratory quotient remains unchanged. Liver slices show a decrease in the ability to oxidize pyruvate (a decrease of 40 per cent) and succinate (30 per cent) after adrenalectomy. The ability of the liver slices from both normal and adrenalectomized rats to oxidize added dextrose is slight. The respiratory depression is less when the operated animals are given salt in the drinking water.

The Q_{O_2} of liver slices from partially-starved, otherwise normal rats is decreased as compared with that of well-fed normal controls. The oxidation of pyruvic acid and of succinic acid shows little dependence on the state of nutrition of the animal.

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EFFECT OF ABLATING THE PYRIFORM-AMYGDALOID AREAS AND HIPPOCAMPI ON POSITIVE AND NEGATIVE OLFACTORY CONDITIONED REFLEXES AND ON CONDITIONED OLFACTORY DIFFERENTIATION[1]

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The effects of extirpating the motor cortex, the prefrontal areas, the hippocampi and large portions of the occipito-parieto-temporal lobes exclusive of the pyriform areas and amygdaloid nuclei on acquiring and acquired olfactory conditioned reflexes has been reported previously. This, a continuation of the general olfactory problem, is concerned with the removals listed in the title on conditioned olfactory reflexes. All animals were subjected to the following tests: *a*, establishment of a conditioned foreleg response to clove vapor; *b*, ability to transfer this reflex to the opposite foreleg; *c*, ability to establish an absence of foreleg response to asafetida and differentiation between two olfactory conditioned reflexes, which means the dog must decide in 7 seconds whether to respond positively or negatively to these vapors; *d*, ability when blindfolded to go to a certain pan by smell and select and open a paper package containing meat from 3 paper packets of like size and texture.

Procedure. The apparatus used for recording the results, the mode of giving the inhalations, the anatomical and physiological controls used, together with a classification of the vapors as to whether they were affective over the olfactory nerve or over both the olfactory and trigeminal, have been fully recorded in earlier papers.

Operations. All extirpations were made from the temporal approach, usually one side at a time, at intervals of about two weeks.[2] Two methods were used for eliminating the pyriform-amygdaloid areas. 1. It was accomplished by use of a specially bent spatula or a loop of steel wire. 2. Utilization of a previous finding that the parieto-occipital cortex was not essential for any of the factors entering into this problem, a dorso-ventral slit was made through the gyrus suprasylvius to the depth of the

[1] Technical assistance for this work was financed by the John and Mary R. Markle Foundation.

[2] The writer is very grateful to the Eli Lilly Co. for supplying without cost the sodium amytal used for anesthesia.

hippocampus. The handle of the scalpel was then passed ventrad along the outer surface of the hippocampus until it hit the base of the skull. Upon moving the tip forward, to the side and backward this area was either completely removed or there remained medially a narrow longitudinal strip of pyriform-amygdaloid area which was functionless. All that is essential for this problem is severance of all fiber connections from this area to isocortex (non-olfactory cortex) and to lower levels. Method 2 proved very satisfactory and was used for all dogs listed in this series except no. 4. If the hippocampus was also to be removed, the ventral half was extirpated with a small hooked knife and the dorsal half by a spatula.

Pyriform amygdaloid ablations. Complete removal of the pyriform lobes or areas without injury to the amygdaloid nuclei or their connecting fibers to the isocortex is very difficult if not impossible. By this area is meant an important olfactory complex (previous paper, fig. 2) consisting of the pyriform lobes and the cortical or most lateral of the amygdaloid nuclei.

Effect on dogs previously conditioned. It can be stated that before operating every dog of this group was able to respond correctly to cloves and asafetida 90 per cent of the time or better irrespective of whether the sequence was 5 cloves to 1 asafetida, 3 to 1, 1 to 1 or 1 to 5. In all dogs failure to respond to cloves was punished by shock. For asafetida the procedure differed—an absence of response in dogs 1 to 4 was rewarded by withholding the shock; while the positive responses in dogs 5 to 9 were punished by whipping, cuffing or scolding depending on the irritability of the dog.

A summary of the more important data obtained from this group of dogs after operating appears in table 1. After unilateral extirpations column 1 of the table discloses that all of the dogs but no. 4 differentiated conditionally between cloves and asafetida. All but dog 4 were also able to differentiate correctly between cloves and anise or xylol.

After bilateral extirpations, columns 2 and 3 of table 1 reveal no especial delay in the appearance or establishment of the positive response to cloves. In most instances it came with the first or second trial. Columns 4 to 8 show that bilateral ablation of the pyriform-amygdaloid area eliminates all olfactory conditioned differentiation with the first foreleg tested for at least 7 seconds when the clove and asafetida vapors were given in the same and even more varied orders of sequence than were used before operating. Ordinarily both clove and asafetida tests ran very strongly positive, the ratio of positive to negative responses being about the same for either vapor. This means for cloves that the ratio of responses to no responses is about the same as before operating; while for asafetida this ratio is practically reversed. Dog 8, however, revealed a

considerable number of failures to respond for both cloves and asafetida, but the ratio of positives to negatives is about the same for each. Con-

TABLE 1

Effect of ablating the pyriform areas and amygdaloid nuclei on acquired olfactory reflexes

DOG	DIFFERENTIATION AFTER FIRST EXTIRPATION	AFTER BILATERAL EXTIRPATIONS											
		First foreleg							Second foreleg				
		First clove response came with test number	Clove reflex established with test number	Differentiation tests					Differentiation tests				
				Number of clove reflexes positive	Number of clove reflexes negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates	Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates
1	Yes	1 L	1	287	15	81	7	No	141 R	10	37	2	No
2	Yes	1 L	1	295	13	60	4	No	23 R	2	5	0	No
3	Yes	1 R	4	72	7	13	1	No	50 L	4	6	8	Poorly
4	No	31 R	42	93	7	16	2	No	47 L	3	13	1	No
5	Yes	2 L	2	195	12	57	7	No					
After several series of asafetida tests				84	18	26	3	No					
6	Yes	2 R	2	189	11	61	4	No	43 L	8	3	19	Yes
After several series of asafetida tests				90	6	28	2	No					
After differentiation was established in left leg		7 R	16	100	0	18	0	No					
7	Yes	3 R	5	309	32	87	8	No					
8*	Yes	1 L	1	148	65	33	16	No					
9	Yes	1 L	1	217	35	70	18	No	50 R	0	10	0	No

R or L denotes order in which forelegs were tested.

* Dog died from exposure.

cerning the second foreleg tested, columns 9 to 13 show dog 6, still able to differentiate excellently with the left foreleg, the ratio of clove positives

to negatives being 43 to 8 and for asafetida 3 to 19. To determine if this dog was still unable to differentiate with the first foreleg tested, several series of differential tests were run off with this (right) foreleg, which resulted (table 1, bottom row for dog 6) in a total inability to hold back its foreleg responses for asafetida and discloses even less ability to differentiate than exhibited in the first tests (top row for dog 6). There is also the possibility that dog 3 was able to differentiate poorly with the second (left) foreleg, for columns 9 to 12 show the ratio of cloves to be 50 to 4 and for asafetida 6 to 8. It is of especial importance to record here that a later section on the brains of this group of dogs shows the contralateral ablations incomplete on these two dogs which differentiated with one foreleg.

All of the dogs which were unable to differentiate conditionally between cloves and asafetida after extirpation of the pyriform-amygdaloid areas were likewise unable to refrain from responding positively to other olfactory stimulating vapors such as anis and xylol when these were substituted for asafetida in differential tests with cloves. It should also be noted that bilateral removal of the pyriform-amygdaloid areas in no way interfered with the dog's ability to transfer the conditioned clove response from one foreleg to the other.

After being unable to obtain conditioned olfactory differentiation with the first foreleg tested following bilateral extirpation of the pyriform-amydgaloid areas, dogs 5 to 9 were subjected to the procedure instigated in a previous study of the prefrontal areas. This consisted of attempts to first reëstablish the negative conditioned reflex by giving only asafetida inhalations and punishing each positive response. If this reflex could be established then additional differential tests would be undertaken.

It will be seen from these tests, as in the preceding ones on the prefrontal areas, that the temperament of the dog played an important rôle on the ability of the dog to refrain from responding to asafetida. Dog 7 of the cocker spaniel variety typifies a group that was high spirited, quick of response and after the operations invariably responded positively to asafetida in the differential tests in spite of severe punishment. Out of 205 consecutive tests made on this dog for asafetida, only 4 were negative and no two came in succession. Although further differential tests were not warranted for this dog, a few were made, using cloves against asafetida or anis or xylol and resulting in only positive responses for each vapor. Dog 5, a shepherd, illustrates another type, which while equally alert was strongly inhibited by the light punishment inflicted for positive responses to asafetida. This type readily acquired a negative response to asafetida when this vapor was tested by itself. In dog 5 the first absence of response came with the 7th trial and from then on was fairly regular but not perfect until the 45th trial. In the tests for differentiation which followed, the first trial for cloves and two others out of the first five were

positive, the first asafetida and the following clove tests were both negative. A summary of these tests (table 1, lower column for dog 5) shows 84 positives and 18 negatives for cloves and for asafetida 26 positives to 3 negatives, which demonstrates a greater ratio of failures to respond for cloves than for asafetida. Dog 6, a greyhound, had more difficulty than dog 5 in regaining the absence of response to asafetida when tested in series, but in the differential tests which followed (table 1, lower column for dog 6) there were proportionately about the same number of positive responses for asafetida as for cloves.

An attempt was made in several of these dogs to differentiate between cloves positively and acetic acid (chiefly an irritant) negatively with little success, which suggests that the general cutaneous fibers to the cortex were severed in the operations.

After learning that a paper package might contain meat these dogs were able when blindfolded to go to a pan and instantly select a paper package containing meat from 3 paper packages of like size and consistency. Their responses to solutions of sugar, salt, quinine and acetic acid dropped on their tongues were the same as normal dogs.

A summary of the autopsy records and studies made on formalin prepared brains of this group of dogs often revealed the presence of a narrow median longitudinal strip of pyriform-amygdaloid cortex, which ranged in width from 2 to 3 mm. in some instances to 4 and 5 mm. in others. In dog 9 the remnant on the right side was 5 to 6 cm. wide. These remnants were usually yellow in color, sometimes of soft texture and all fiber connections to the isocortex were severed. Histological sections made of several of them indicated incapability of functioning. A complete removal of the pyriform-amygdaloid area was accomplished on the left side of dogs 2, 3, 6 and 9 and on the right side of dogs 4 and 8.

Dogs 3 and 6 which had the pyriform-amygdaloid areas completely extirpated from the right side possessed median longitudinal remnants of considerable width on the left side. In dog 3, the pyriform cortex was intact with the isocortex caudally, but severed elsewhere. In dog 6, the longitudinal remnant was 8 to 9 mm. wide and connected with the isocortex cephalad and caudad. Histological sections of both remnants revealed many normal cells in the regions which were connected to the isocortex. The presence of these large remnants of the right pyriform areas and their probable connections with the isocortex in dogs 3 and 6 can readily explain how these two dogs were able to respond correctly at times with their left legs to cloves and asafetida. The anterior commissure of dog 4 was sectioned after the Marchi method and found to be filled with degenerated fibers, but the same may be said of other dogs which were able to differentiate correctly after unilateral extirpations of the opposite pyriform-amygdaloid area.

With the exception of dog 1, paralyzed in the right leg, there was only

slightly more or no more than normal degeneration in Marchi sections of the pyramids. There was always more than the normal amount of degenerated fibers in the fornices of Marchi sections through the hypothalamus.

Effect on conditioning. The procedure was identical to that used in the previous section except that removal of the pyriform-amygdaloid areas preceded all attempts at conditioning. Table 2 summarizes the effects of the lesions on three of the tests that were used on the animals previously conditioned. Columns 1 and 2 of this table show that these dogs experienced little, if any, difficulty in acquiring the positive conditioned response for cloves. The apparent delay in dog 12 for the first foreleg tested can readily be explained by the fact that this leg was paralyzed, but when established the reflex became very regular. Columns 8 and 9

TABLE 2

Effect of ablating the pyriform areas and amygdaloid nuclei on acquiring olfactory conditioned reflexes

	FIRST FORELEG TESTED (RIGHT)							SECOND FORELEG TESTED (LEFT)						
			Differentiation tests							Differentiation tests				
DOG	First clove response came with test number	Clove reflex established with test number	Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates	First clove response came with test number	Clove reflex established with test number	Number of clove responses positive	Number of clove responses negative	Number of asafetida responses positive	Number of asafetida responses negative	Differentiates
10	36	75	288	13	78	6	No	20	98	150	0	30	0	No
11	12	31	266	34	54	6	No	9	37	24	1	5	0	No
12	48	257	116	9	20	0	No	31	32	100	0	20	0	No

Dog 12 paralyzed in right foreleg.

indicate a few more than the normal number of tests were required to transfer the clove reflex from one foreleg to the other. It is apparent from columns 3 to 7 and 10 to 14 that these dogs were unable to acquire an absence of response with either foreleg for asafetida when mixed with clove inhalations in ratios of 5 cloves to 1 asafetida, 3 to 1, 1 to 1 or 1 to 5. It made no difference whether the ablations were made in one operation, dog 10, or in two operations, dogs 11 and 12.

The autopsy records and studies made from formalin prepared brains revealed the presence of median longitudinal strips of pyriform-amygdaloid cortex ranging in width from 1 to 2 mm. to 3 to 5 mm. These remnants had a yellow color and all connections with the isocortex were severed. Histological sections of the remnants of dogs 11 and 12 demonstrated that the cells of the lobe and nucleus were incapable of functioning.

Marchi sections through the medulla showed complete degeneration of the fibers of the left pyramid of dog 12 but insufficient in the other pyramids to block impulses. Marchi sections through the hypothalamus revealed slightly more than the normal number of degenerated fibers in the fornices.

All 3 dogs were able to select a paper package containing meat from 3 paper packages of like size and texture. Their taste responses were normal to solutions of sugar, salt, quinine and acetic acid dropped on their tongues.

PYRIFORM-AMYGDALOID AND HIPPOCAMPAL EXTIRPATIONS. Although an earlier report demonstrated that the hippocampi could be removed without

TABLE 3

Effect of ablating the pyriform areas, amygdaloid nuclei and hippocampi on acquired olfactory reflexes

DOG	DIFFERENTIATED BEFORE OPERATIONS	AFTER LEFT SIDE EXTIRPATIONS			AFTER RIGHT SIDE EXTIRPATIONS						
							Differentiation tests				
		First clove response came with test number	Clove reflex established with test number	Differentiates	First clove response came with test number	Clove reflex established with test number	Number of clove reflexes positive	Number of clove reflexes negative	Number of asafetida reflexes positive	Number of asafetida reflexes negative	Differentiates
13	Yes	25	51	Yes	1	1	194	8	36	4	No
	R	R			R						
14	Yes	1	1	Yes	1	1	166	27	42	10	No
	R	R			R						
15	Yes	3	3	Yes	1	1	71	9	28	3	No
	R	L			L						

R or L signifies the foreleg tested.
Right foreleg of 13 was paralyzed.

affecting the positive clove response or the correct responses for cloves and asafetida when mixed in various ratios and the previous section showed that eliminating the pyriform-amygdaloid areas did not interfere with the clove reflex it seemed possible that deleting both of these important anatomical areas for olfaction might block or seriously interfere with the positive conditioned response for cloves. This was accomplished in 3 dogs, using the second method for eliminating the pyriform-amygdaloid areas.

Effect on acquired reflexes. Column 1 of table 3 indicates that before operating dogs 13 to 15 could respond correctly 90 per cent of the time to cloves and asafetida in 7 seconds when the ratio of inhalations was 5 cloves to 1 asafetida, 3 to 1, 1 to 1 and 1 to 5. All clove errors were punished by

shock and all asafetida by a whip or cuff. Columns 2 and 3 reveal that the same ability to respond correctly to cloves and asafetida continued after a contralateral ablation for dogs 13 and 14 and a homolateral ablation for dog 15. The delayed appearance of the reflex in dog 13 was caused by paralysis.

After bilateral ablations it is obvious from columns 5 and 6 of table 3 that the second lesion, made on the right side, in no way affected the clove response. It came with and became constant with the first trial. As was to be expected from the previous experiments their ability to differentiate correctly between cloves and asafetida in 7 seconds was completely lost. Since dog 15 never made any unnecessary movements and his responses to both cloves and asafetida were more deliberate than dogs 13 and 14 he was given an additional series of differential tests after having quickly obtained an absence of response to asafetida when this vapor was tested continuously. The second differential tests were identical to the first in showing no signs of conditioned differentiation.

After learning that a paper package might contain meat all of these dogs were able when blindfolded to find a pan and select from it a paper package containing meat from 3 paper packets of like size and texture. Their taste responses to solutions of sugar, salt, quinine and acetic acid dropped on their tongues were unchanged after the lesions.

Autopsy and examination of formalin prepared brains of this group demonstrated complete removal of the pyriform-amygdaloid area and hippocampus from the right side of dog 15. There remained on both sides of dogs 13 and 14 median longitudinal strips of the pyriform-amygdaloid cortex 2 to 5 mm. wide. There was also a similar remnant on the left side of dog 15. All of these remnants were yellow in color or soft in texture and all connections with the adjacent isocortex were obviously lost. There can be no question but that the hippocampi were also functionally eliminated. In dogs 13 and 14 and on the left side of dog 15 about 5 mm. of the extreme ventral tips of these structures remained at autopsy, which means that about 90 per cent including the fornix was removed, and of the remnant at least one-half was yellow in color.

Marchi sections of the medulla revealed very little more than the normal number of degenerated fibers in the pyramids, except in the left of dog 13, which would account for the paralysis of the right leg.

Discussion. The complicated anatomical relationships of the central olfactory system as worked out by Cajal, van Gehuchten, Johnston and others and shown in figure 2 of the previous paper can be summarized as follows: 1. Olfactory impulses entering the olfactory bulbs are distributed to 3 primary areas. 2. These areas supply a primitive median component, the hypothalamus and a more lateral component, the pyriform-amygdaloid complex or area. 3. The hypothalamus furnishes the chief

olfactory reflex connections to lower levels and the pyriform-amygdaloid area the chief afferent olfactory connections to the isocortex (general or non-olfactory cortex). Attention, however, is called to one significant exception originating from the hypothalamus, namely, the mammillothalamic bundle which sends many fibers to the anterior thalamic nucleus where olfactory impulses may be relayed to the cerebral cortex.

Concerning the evolution of the olfactory system Johnston has clearly shown that the pyriform lobes and the most lateral of the amygdaloid nuclei (cortical nucleus) have developed late phylogenetically as a probable result of increased needs from a changed environment from water to land. Johnston calls attention to the interconnections between the lateral portion of the amygdaloid nucleus and the pyriform lobe and from these areas the isocortex of the same and opposite sides; the latter were said to be short and long association fibers, external capsule fibers and anterior commissure fibers. He ventured the opinion that the pyriform lobe and the lateral or cortical amygdaloid nucleus are associated with olfactory-somatic correlation and the more median amygdaloid nuclei are associated with olfactory-gustatory correlation.

The earlier literature on the effects of decortication and temporal lobectomy on olfaction have been considered in earlier papers. More recently Dusser de Barenne, and Bard and Rioch have reported olfactory behavior after removals of the cerebral cortex; while Klüver and Bucy have noted strong oral tendencies (licking, chewing, touching of the lips, "smelling") after temporal lobectomy.

The writer agrees with Papez that no (recent) evidence has been presented to demonstrate that the hippocampi are important structures for olfaction. However, none of the writer's dogs with deleted hippocampi showed any signs of having lost a "central emotive center" as postulated by Papez in 1937.

Unfortunately the writer has been unable to see some of the early Russian work on olfactory conditioned reflexes, but according to Zavadsky's review it cannot be taken seriously. Zavadsky's contribution consisted of removing the pyriform lobes and some of the adjacent hippocampus and isocortex from 2 dogs. He afterward obtained a conditioned salivary reflex from inhaling camphor and reflex movements of the nostrils and secretions of saliva from the smell of meat powder. Zavadsky like several others was unfortunate in the selection of his olfactory stimulating vapor, for the writer demonstrated (1937) that a conditioned reflex could be obtained from camphor after the olfactory nerves had been cut and is consequently a trigeminal as well as an olfactory stimulant.

It is obvious from this investigation that a positive conditioned foreleg response to clove vapor is readily acquired and not lost from bilateral elimination of the pyriform areas, amygdaloid nuclei and 95 to 100 per cent

of the hippocampi and fornices. The real effects from these bilateral extirpations came with the conditioned olfactory differential tests, when it was shown that removal of the pyriform-amygdaloid areas with or without the hippocampi prevented correct conditioned differentiation in 7 seconds between cloves and asafetida or cloves and anis or xylol when clove vapor was given to the others in ratios of 5 tests to 1, 3 to 1, 1 to 1, and 1 to 5. These results were the same whether the tests followed or preceded and followed the operations, or whether the removals were made in one or two operations.

As reported for deletion of the prefrontal areas the temperamental differences of the dogs played a considerable rôle in the character of the responses after extirpation of the pyriform-amygdaloid areas. This was especially noticeable in the serial tests for obtaining absence of response to asafetida. In spite of severe punishment the high spirited dogs continued to respond positively to asafetida, while the more quiet dogs readily acquired an absence of response, but when asafetida tests were afterward mixed with clove, there was no sign of conditioned olfactory differentiation between these vapors with either group.

Two observations have come out of this study in non-support of the pyriform-amygdaloid-hippocampal areas being the centers or at least the sole centers for the detection of minute differences in odors. They are: 1, after the operations these dogs were able by smell to select a paper package containing meat from 3 paper packets of like size and texture; 2, following an erroneous response to cloves or asafetida they may brace themselves or cry as if in expectancy of punishment that is to follow.

Brown and Ghiselli have recently reported olfactory discrimination to be subcortical in rats, but as was previously pointed out (1938) for Swann's work, they used an olfactory stimulating vapor against creosote, an irritant, effective over both the trigeminal and olfactory nerves.

It was first supposed after finding the Marchi sections of the anterior commissure of dog 4 (only dog not able to differentiate conditionally after unilateral deletion of the pyriform-amygdaloid area) full of degenerated fibers that severance of this bundle might have blocked all of the crossed afferent impulses in this dog and thus prevented conditioned olfactory differentiation in the contralateral leg. However it was found later that Marchi sections of the anterior commissure from other unilateral lesion dogs, which differentiated conditionally, were also filled with degenerated fibers.

Since deletion of the pyriform-amygdaloid areas produced the same interference with conditioned olfactory differentiation as resulted from removal of the prefrontal areas, their anatomical relationships would suggest that the pyriform-amygdaloid extirpations cut off the normal afferent supply of olfactory impulses to the cortical areas concerned with

conditioned olfactory differentiation in 7 seconds, a very high order of olfactory synthesis.

Likewise from the same order of reasoning the complete series of studies suggests that the afferent olfactory impulses which go to the isocortex concerned with the production of a conditioned olfactory response ordinarily travel by way of the mammillo-thalamic bundle, anterior thalamic nucleus and internal capsule.

All that can be claimed for these and the previous olfactory studies is that they represent the normal mode of operation during the interval of experimentation after operating, which varied from 6 weeks to 4 months. It is possible that the isocortex may possess inactive areas or have other olfactory connections which may at some future time take over the functions of the destroyed areas.

SUMMARY

To determine the effects of certain temporal lobe lesions on olfaction' the following tests were made after and before and after the operations: *a*, establishment of a conditioned foreleg reflex to clove vapor; *b*, transference of the clove reflex from one foreleg to the other; *c* establishment of a negative conditioned reflex (absence of foreleg response) for asafetida and conditioned differentiation, which involved a decision in 7 seconds whether to respond positively or negatively to the vapor inhaled, irrespective of any order in which it might come; *d*, food from no food discrimination by smell.

With one exception, considered in the discussion, unilateral extirpation of the pyriform-amygdaloid areas produced little or no effect on *a*, *b*, *c* or *d* with either the contralateral or the homolateral foreleg.

Bilateral extirpation of the pyriform-amygdaloid areas or severance of their isocortex connections had little or no effect on *a*, *b* or *d*, but abolished *c* in every instance in which elimination was complete.

The inclusion of the hippocampi (95 to 100 per cent) to the pyriform-amygdaloid ablations resulted in no additional effects on *a*, *b* or d.

Other purely olfactory vapors such as anis and xylol were substituted for asafetida in the differential tests with the same results.

Consideration was given in the discussion to: 1, the relationship of the pyriform-amygdaloid areas to the afferent side of conditioned olfactory differentiation; 2, certain temperamental variations noted in *c*; 3, the likelihood of the mammillo-thalamic bundle furnishing important afferent olfactory impulses for a conditioned reflex.

Deletion of the pyriform-amygdaloid-hippocampal areas produced no effect on the taste responses elicited from solutions of sugar, salt, quinine and acetic acid dropped on the tongue.

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CIRCULATION TIME THROUGH AERATED AND ATELECTATIC LUNGS IN DOGS AS DETERMINED BY THE USE OF SODIUM CYANIDE

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Stewart's formula (1) V equals $Q\,\frac{60}{T}$, which concerns the pulmonary circulation, may be applied to the circulation through one lung. Thus V' equals $Q'\,\frac{60}{T'}$ where V' is the minute volume flow of blood through one lung; Q' is the quantity of blood in one lung; and T' is the mean blood velocity through that lung in seconds. V' and Q' are decreased during atelectasis (2, 3, 4) but no report was found in which both V' and Q' had been determined in the same atelectatic lung thus permitting calculation of T'. Similarly, no direct study of the effect of atelectasis on the mean blood velocity through a lung was encountered in the literature. An index of the mean velocity of blood flow through the lungs may be obtained by measuring pulmonary circulation time (1, 5). In this study the circulation time through aerated and atelectatic lungs and through certain associated vascular circuits was determined by the use of sodium cyanide (6, 7).

Material and procedure. Full grown healthy dogs were used. Under pentobarbital (Nembutal) anesthesia the chest was opened through the left fourth intercostal space. Throughout the experiments the intermittent intratracheal insufflation was adjusted to a minimum compatible with the abolition of spontaneous respiratory movements. The pericardium was opened and a loop of catgut was passed around the left branch of the pulmonary artery so that the right and left branches of the pulmonary artery could be supported while injections were being made into them. The cardiac rate and the rate of the respirator were determined with a stopclock.

Two series of observations were made, the first while both lungs were being aerated, and the second after atelectasis of the left lung had been caused by division and closure of the bronchus. In each series, adequate doses of sodium cyanide (0.18–0.25 c.c. of 0.5 per cent solution) were in-

jected into 1, the right branch of the pulmonary artery (R.B.P.A.); 2, the left branch of the pulmonary artery (L.B.P.A.); 3, the right ventricle (R.V.), and 4, the left ventricle (L.V.). The interval between the injection of sodium cyanide and the occurrence of the end point was determined by a stopclock, and it represents the circulation time from the point of injection to the carotid sinus (8, 9). From these results the circulation time through the lungs[1] was calculated.

RESULTS. When both lungs were aerated by intermittent insufflation (table 1), the results were as follows: the L.B.P.A. to carotid sinus (C.S.)

TABLE 1

Circulation time from site of injection to carotid sinus and the calculated pulmonary circulation time through the right and left lungs when both are aerated

	TRIAL	CARDIAC RATE PER MINUTE	RESPIRATOR RATE PER MINUTE	CIRCULATION TIME FROM SITE OF INJECTION TO CAROTID SINUS IN SECONDS				CIRCULATION TIME THROUGH RIGHT LUNG	CIRCULATION TIME THROUGH LEFT LUNG
				L.B.-P.A.	R.B.-P.A.	R.V.	L.V.		
Experiment 1, 4/20/37	1	140	15	9.0	9.5	10.0			
Experiment 2, 8/11/37	1	114	26	9.5	9.5	11.5	5.0	4.5	4.5
	2	114	26	8.5	8.0	12.0	5.0	3.0	3.5
	3	110	26	9.5	8.5	10.0	4.5	4.0	5.0
Experiment 3, 8/25/37	1	126	30	10.0	9.0	10.0	5.0	4.0	5.0
	2	120	30	10.0	10.0	10.0	5.0	5.0	5.0
	3	120	30	10.0	10.0	10.0	5.0	5.0	5.0
Experiment 4, 4/15/37	1	124	36	10.0	8.5				
Average...........		121	27	9.5	9.1	10.5	4.9	4.2	4.6

L.B.P.A. = Left branch pulmonary artery. R.B.P.A. = Right branch pulmonary artery. R.V. = Right ventricle. L.V. = Left ventricle.

circulation time varied from 8.5 to 10 seconds with an average of 9.5 seconds. The R.B.P.A. to C.S. circulation time was only slightly less, ranging from 8 to 10 seconds and averaging 9.1 seconds. The circulation time for the vascular segment from the R.V. to the C.S. varied from 10 to 12 seconds with an average of 10.5 seconds. The L.V. to C.S. circulation time averaged 4.9 seconds. The pulmonary circulation time through the right and left lungs was obtained by subtracting the L.V. to C.S. circula-

[1] The term, "circulation time through the lungs," used here is defined as the time calculated to be necessary for the passage of blood from either branch of the pulmonary artery to the left ventricle.

TABLE 2

Circulation time from the sites of injection to the carotid sinus and the calculated pulmonary circulation time through the right and left lungs when the right lung is aerated and the left lung is atelectatic

	TRIAL	TIME	CARDIAC RATE PER MINUTE	RESPIRATOR RATE PER MINUTE	CIRCULATION TIME FROM SITE OF INJECTION TO CAROTID SINUS IN SECONDS				NET CIRCULATION TIME THROUGH FUNCTIONING LUNG	NET CIRCULATION TIME THROUGH NONFUNCTIONING LUNG	REMARKS
					L.B.P.A.	R.B.P.A.	R.V.	L.V.			
4/20/37: Experiment 1. Sex, male; weight, 22.7 kgm.; anesthetized with 25 cc. pentobarbital solution, 1:45 p.m.	1	3:52	120	16	12.0	8.5	9.5	4.0	4.5	8.0	Lung collapsed and mottled
	2	4:03	128	20	12.0	7.0	7.0	5.0	2.0	7.0	
	3	4:13	112	24	10.5	6.5	7.5	5.0	1.2	5.5	
	4	4:30	100	24	10.0	7.0	9.0	5.0	2.0	5.0	Lung purple
Average for group			115	21	11.1	7.25	8.25	4.75	2.4	6.3	
8/11/37: Experiment 2. Sex, female; weight, 17.4 kgm.; anesthetized with 18 cc. pentobarbital solution at 12:45 p.m.	1	2:32	120	32	14.0	10.0	10.0	5.0	5.0	9.0	Lung collapsed; central portion "liver-like"
	2	2:45	138	24	12.5	9.0	10.5	5.0	4.0	7.5	
	3	2:55	128	30	11.0	8.0	10.5	5.0	3.0	6.0	
	4	3:33	126	30	14.5	9.5	8.5	5.0	4.5	9.5	Lung entirely "liver-like"
	5	3:40	120	30	14.0	9.0	10.0	5.5	3.5	8.5	Lung entirely "liver-like"
Average for group			126	29.2	13.2	9.1	9.9	5.1	4.0	8.1	
8/25/37: Experiment 3. Sex, male; weight, 30.3 kgm.; anesthetized with 31 cc. pentobarbital solution at 12:45 p.m.	1	2:48	120	36	10.0	8.0	8.5	5.0	3.0	5.0	Lung collapsed; slight crepitus in peripheral portions
	2	3:35	136	30	9.0	7.5	8.0	4.5	3.0	4.5	
	3	3:45	134	36	11.0	8.0	8.0	5.0	3.0	6.0	Lung "liver-like"
	4	4:05	114	36	10.5	8.0	9.0	5.0	3.0	5.5	Lung "liver-like"
	5	4:15	132	36	11.5	8.0	10.0	5.5	2.5	6.0	Lung "liver-like"
	6	4:40	114	36	10.5	8.0	10.0	5.5	2.5	5.0	Lung "liver-like"
Average for group			125	35	10.4	7.9	8.9	5.09	2.83	5.3	
4/15/37: Experiment 4. Sex, male; weight, 24.3 kgm.; anesthetized with 25 cc. pentobarbital solution at 2:00 p.m.	1	3:10	132	48	11.5	6.5	7.0	5.0	1.5	6.5	Lung collapsed; central portion heavily mottled
	2	3:25	136	40	10.0	6.5	7.0	4.5	2.0	5.5	
	3	3:55	138	42	10.0	6.5	7.0	5.5	1.0	4.5	
	4	4:22	138	42	10.0	7.0		5.0	2.0	5.0	Lung "liver-like" throughout
Average for group			136	43	10.4	6.6	7.0	5.0	1.6	5.3	
Total average			125.5	32.2	11.2	7.8	8.7	5.0	2.8	6.2	

L.B.P.A. = left branch pulmonary artery. R.B.P.A. = Right branch pulmonary artery. R.V. = Right ventricle. L.V. = Left ventricle.

tion time from the results obtained by injections into the branches of the pulmonary artery. The average circulation time through the right lung was 4.2 seconds, and the average circulation time through the left lung was 4.6 seconds, or 0.4 second longer.

Table 2 shows the results obtained after atelectasis of the left lung had been produced by division of its bronchus, closure of the divided ends, and subsequent absorption of the contained gases. The L.B.P.A. to C.S. circulation time ranged from 9 to 14.5 seconds, with an average of all these determinations through this circuit (part of which was composed of the atelectatic lung) of 11.2 seconds. The R.B.P.A. to C.S. circulation time varied from 6.5 to 10 seconds, with an average of 7.8 seconds. The R.V. to C.S. circulation time ranged from 7 to 10 seconds with an average of 8.7 seconds. The L.V. to C.S. circulation time in this series varied from 4 to 5.5 seconds and averaged 5 seconds.

By subtracting the L.V. to C.S. circulation time from the results obtained by injections into the branches of the pulmonary artery the pulmonary circulation time through the right and left lungs was obtained. The figures for the circulation time through the aerated lung varied from 1 to 5 seconds, and averaged 2.6 seconds. For the atelectatic lung the extremes of circulation time were 4.5 and 9 seconds, with an average of 6.2 seconds, or 3.4 seconds longer than the circulation time through the aerated lung.

Discussion. Only two reports dealing with the pulmonary circulation time in dogs were found in the literature. Stewart (1) found the pulmonary circulation time in intact anesthetized dogs to average 8 seconds. He obtained this result by subtracting the left ventricle to femoral artery circulation time from the right ventricle to femoral artery circulation time. Kuno (10) using a canine heart-lung preparation found that the mean pulmonary circulation time averaged 2.8 seconds in five experiments. He called this interval the pulmonary artery to pulmonary vein circulation time. Because of the differences in the vascular segments studied and in experimental conditions and methods, no direct comparison can be made between the results of Stewart or Kuno and those presented in this report.

The possibilities of 1, inaccuracies in timing; 2, variations in the degree of atelectasis of the left lung, and 3, irregularities in the rate and extent of aeration of the right lung must be considered in these experiments. The end points caused by adequate doses of sodium cyanide are clear-cut even under anesthesia and continue to be so after repeated determinations. Errors in timing from this source therefore need not be considered. The relation of the time of injection to the phases of the cardiac cycle constitutes a source of error which becomes less important with increases in cardiac rate. With a cardiac rate which ranged between 100 and 138 per minute, the maximal delay in the right and left ventricles could not prolong the R.V. to C.S. circulation time more than approximately a

second. The remaining three determinations were subject to a maximal prolongation of approximately a half-second, due to delay of the sodium cyanide in the left ventricle. Calculation of the circulation time through the lungs by subtracting the L.V. to C.S. circulation time from the circulation time determined by injection into the branches of the pulmonary artery may be subject to no error if the delay in the left ventricle prolongs the two sets of determinations to the same extent. The maximal error (approximately one-half second) in the calculated pulmonary circulation time would occur when the greatest possible delay in the left ventricle affected one determination and when there was no delay in the left ventricle to affect the other determination. Stewart (1) has pointed out that when a mean of a number of successive determinations is taken, the error due to delay in the ventricle or ventricles is automatically approximated to the average. Errors in timing due to the human element, if present, were small, as is indicated by the uniformity of the results in the L.V. to C.S. circulation time determinations, in which the effects of other variables were minimal.

A comparable degree of atelectasis of the left lung in all four animals was assured by the length of the intervals (thirty to forty-five minutes) between closure of the bronchus and the first determinations of the circulation time shown in table 2. The fact that 79 per cent of the values for the L.B.P.A. to C.S. circulation time fell within plus or minus ten per cent of the average value of 11.2 seconds is further assurance that no great variation in the degree of atelectasis was present.

The wider range of figures for the R.B.P.A. to C.S. circulation time is probably due to several variable conditions such as the respirator rate, the degree of expansion of the right lung, and the length of time the increased intratracheal pressure was maintained, this latter situation being dependent on the proportion between the inside diameter of the trachea and the outside diameter of the intratracheal tube.

From the results of the experiments described here, it is seen that in a lung recently rendered atelectatic by the occlusion of its bronchus, the values for V' (the minute flow of blood through one lung) and Q' (the quantity of blood in one lung) are such as to consistently cause a decrease in T' (the mean blood velocity through the lung) as indicated by the pulmonary circulation time. Conversely, when one lung has been rendered atelectatic, the minute volume flow of blood and the quantity of blood in the other lung are such that the circulation time through the aerated lung is consistently decreased.

SUMMARY

1. By the injection of adequate doses of sodium cyanide into 1, the left branch of the pulmonary artery; 2, the right branch of the pulmonary artery; 3, the right ventricle, and 4, the left ventricle, the circulation time

from these sites of injection to the carotid sinus was determined in dogs with open chests, before and after division and closure of the left bronchus. From these results, the circulation time through the right and left lungs was calculated.

2. When both lungs were aerated, there was no great difference in the circulation time through the right and left lungs. The average calculated circulation time for the right lung was 4.2 seconds, and for the left lung it was 4.6 seconds.

3. When one lung was rendered atelectatic by the closure of its bronchus, the circulation time through the atelectatic lung was increased to an average value of 6.2 seconds, whereas the circulation time through the aerated lung was decreased to an average of 2.8 seconds.

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STIMULATION OF NERVES BY DIRECT CURRENTS

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It is generally accepted that the nerve impulses elicited by the make of a direct current originate exclusively at the region of the nerve in contact with the cathode, while the impulses set up by the break of the current start exclusively at the anode. The statement implies two laws, one positive and the other negative, as follows: that the make starts impulses at the cathode and the break at the anode; and that the anode cannot stimulate when the current is closed, and the cathode when the current is broken.

A critical survey of the experimental basis for these laws (see Gotch, 1900, and Laugier, 1921, for summaries of this evidence) shows that, whereas the positive law is adequately supported by the data, the negative statement is based on the inference that stimulation by the make at the anode or by the break at the cathode is an unnecessary assumption—i.e., that the observations adduced in support of that assumption can be reasonably explained by the first law.

In a study of the changes of electrical excitability of nerve produced by applications of direct current (Rosenblueth, 1941) certain observations were made which did not fit reasonably with the classical concepts of nerve stimulation mentioned above. The present report deals with experiments made to test the validity of the negative statement in the classical law of electrical stimulation of nerve.

Method. The nerves studied were the cat's popliteal, peroneal and phrenic. The animals were usually anesthetized with dial (Ciba, 0.75 cc. intraperitoneally). Control experiments made on animals which had been decapitated 30 to 90 minutes previously, under ether, showed that the use of dial anesthesia led to only minor quantitative differences in the results.

The nerves were either *in situ*, with the circulation relatively intact, or else excised and placed in a moist chamber. The indicator of activity of the circulated popliteal nerves was the contraction of the muscles attached to the Achilles tendon. The legs were held firmly by drills inserted into the tibiae. The tendons were attached to a tension myograph recording on a kymograph. Upward excursions in the tracings denote contraction.

In the excised nerves the nerve impulses were recorded electrically from a cathode-ray oscillograph after suitable amplification. The details of the

different modes of recording adopted for different purposes will be described with the corresponding results.

When the popliteal nerves were *in situ* they were severed centrally, at the emergence of the sciatic from the pelvis. The electrodes were chlorided silver wires supported and shielded by split rubber tubing. The popliteal was usually separated from the peroneal at three places in the thigh, for spaces of about 1.5 to 2 cm. The rubber electrode holders, supporting 2 to 4 electrodes, were inserted through these spaces. Cotton was then packed lightly around the electrodes, providing relative insulation from the surrounding muscles. These muscles and the skin were then sewed up, leaving only the insulated leads of the electrodes to emerge. The nerves were therefore at normal temperature and protected from desiccation. Throughout the dissection damage of the blood vessels was carefully avoided.

The electrodes used for applying direct currents to the excised nerves were calomel half-cells. The bridge to the nerve was provided first by agar-Ringer and finally by the wicks, soaked with Ringer, upon which the nerves lay. Tests showed that within the range of voltages employed the electrodes were impolarizable. For recording the nerve responses no special precautions were usually taken with regard to the polarizability of the electrodes.

The two methods employed offered different advantages and disadvantages, respectively. The nerves *in situ* were in quite normal physiological condition. On the other hand, the non-polarizability of chlorided silver wires is only relative. Furthermore, the popliteal nerves as prepared had several points of intimate contact with surrounding tissues, mainly with the peroneal nerve. It is possible, therefore, that in certain observations the currents applied may have entered or left the nerves tested at other points than those in contact with the electrodes used. In the excised nerves the application of the currents could be rigorously controlled, but the physiological condition was unavoidably deeply disturbed.

The sources of d.c. were usually dry cells, the intensity being regulated by means of a potentiometer. It was found that ordinary spring keys are quite satisfactory for making the current—i.e., the difference of potential across the electrodes can be suddenly established and maintained. The release of such a key, on the other hand, was found quite unreliable for breaking the current suddenly, as evidenced by the irregularity of the responses to the opening of the currents. The procedure adopted was, therefore, to make the current by means of a key in the battery circuit ahead of the potentiometer regulating the voltage, and to break it by closing with a similar key a short circuit of negligible resistance placed between the potentiometer and the series resistances, electrodes and nerve.

The voltmeter with which the potential drops were measured was some-

times placed ahead of resistances added in series to the electrodes, or else it read the voltage drop across the electrodes or the nerve. An ammeter was also used sometimes to register the intensity of the currents flowing through the nerve.

In the excised nerves the d.c. was sometimes applied for 1 to 5 seconds with a set-up similar to that just mentioned. For other observations it was found more expedient to use brief pulses applied repetitively. A synchronous motor drove a cardboard circular disk at the rate of 30 revolutions per second. An angular slit with an amplitude of 60 to 180° cut in the disk allowed the intermittent illumination of a photoelectric cell for 5.56 to 16.67 msec., 30 times per second. A careful alignment of the borders of the slit in the disk with those of another slit 1 mm. wide in front of the photoelectric cell tended to render the make and the break of the discharges from the cell quite sudden. The current was made to full value or broken within an interval not greater than 0.2 msec.

The pulses from the photoelectric cell were intensified by one stage of d.c. amplification. A potentiometer regulated the output to the nerve. By changing the balance of the amplifier it was possible to deliver to the nerve small currents in either direction in the intervals between the pulses originated at the photoelectric cell. The use of this mode of stimulation was invaluable for the detailed analysis of the responses of the nerve to d.c. pulses. The sweep circuit of the oscillograph was driven 30 or 60 times per second from the a.c. main, which also drove the motor of the stimulator. An accurate synchronization could thus be obtained and the steady pattern on the face of the tube could be photographed or else the changes brought about by small changes of the voltage of the d.c. pulses could be readily appreciated and evaluated.

Results. A. *The responses of nerve to ascending or descending currents of various intensities.* The observations were made mainly by stimulating through two electrodes on the popliteal nerve and recording the corresponding muscular mechanical responses. A current was delivered for 1 to 15 seconds and the same current was applied 1 to 5 minutes later through the same electrodes in the reversed direction. The effects of a given current were quite repeatable—that is, the responses to a given current were similar whether the application was made early in the experiment or later, after several other stimuli had been applied, provided that very strong currents were not used in the course of the observations.

When series of applications were made with increasingly higher voltages the results were as follows. Threshold was usually the same for both the ascending and the descending currents, provided the region of the nerve tested was sufficiently far away (about 3 cm.) from the cut end. Any difference present was entirely random if several electrodes were tested successively. That is, if the ascending current had a lower threshold than

the descending current with a certain pair of electrodes, changing one or both of these electrodes could lead to the opposite difference, and no evidence was encountered of any consistent effect anywhere along the nerve. The differences, when present, are therefore readily explained by unavoidable dissimilarities in the dissection of the nerve and in the intimacy of the contact of the several electrodes with the nerve.

The responses at threshold were invariably to the make of the currents, indicating that the break is a less effective stimulus than the make (confirming Pflüger, 1859).

As the currents were intensified, and usually before any contractions were obvious to the break of the currents, the responses to the make, which were single twitches at threshold, became tetanic—i.e., higher than a maximal twitch—thus showing repetitive firing by the nerve. In every one of the 43 nerves studied in these conditions such repetitive discharges were seen with quite weak currents (1.5 to 3 times rheobase).

Further intensification of the currents began to reveal marked differences between the effects of descending (i.e., cathode toward the muscle) and ascending (i.e., anode toward muscle) currents. First the tetanus corresponding to the make of the current (Pflüger's tetanus) was greater when the cathode was toward the muscle than in the reverse direction. The difference in the response to the make was due both to an increase for the descending and a decrease for the ascending current, as compared with the effects of weaker currents. Correspondingly, the tetanus at the opening of the current (Ritter's tetanus) was slightly greater when the anode was toward the muscle. With even stronger currents, the tetanus during the passage of the current was greater when the anode was proximal to the muscle than in the reversed direction. The responses to the make of the descending current were at this stage much smaller than previously, while the effects of the ascending application had increased considerably. Conversely, with these strong currents the opening tetanus was greater for the descending than for the ascending direction.

Figure 1 illustrates some of the characteristic steps in a typical series of observations, and the sequence of responses is summarized in table 1. It is important to note that the decrease of the responses to the make of the current when the cathode was toward the muscle (cf. fig. 1B, C, D) was probably not due to block of nerve impulses. Tests were made for block during the passage of the current, by stimulating supramaximally through electrodes placed further away from the muscles than those for the d.c. Only exceptionally was any significant block detected with the intensities of current discussed thus far. Absence of block or only slight partial block with characteristic responses to relatively strong d.c. are illustrated in figure 2.

In some experiments the nerve action potentials were recorded diphas-

ically during the passage of d.c. by means of electrodes placed peripherally. The nerve electrograms were closely parallel to the muscle mechanograms: large contractions were attended by rich bursts of spike potentials, whereas small contractions showed only discrete nerve impulses.

Table 1 differs significantly from Pflüger's (loc. cit.) classical "law of contraction" in two respects. First, the term "twitch," which was used throughout by Pflüger in summarizing his observations, is often substituted by the term "tetanus" in recognition of the fact, emphasized by Pflüger himself, that the responses of nerves to direct currents of intensities

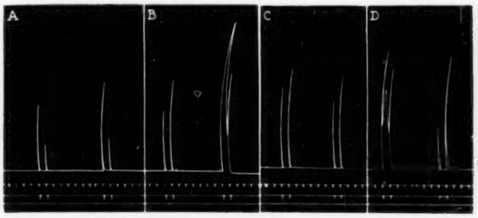

Fig. 1

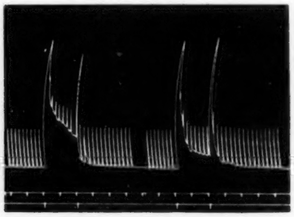

Fig. 2

Fig. 1. Responses of the gastrocnemius-plantaris-soleus muscles to stimulation of the popliteal nerves with various intensities of direct current. Cat under dial. The electrodes were placed toward the middle of the thigh, with an interelectrodal distance of 15 mm. The pairs of signals below the time mark indicate the beginning and end of a period of application of direct current (the same convention was followed in figs. 2, 3 and 4). The make and break responses are arranged in pairs, the direct current having been applied for each voltage first in the ascending direction (anode toward muscle) and then in the descending direction (cathode toward muscle).

The voltages for the successive pairs were: A, 0.5; B, 2.0; C, 5.0; D, 10.0.

In this and the following kymograph records the time signal denotes 5-sec. intervals.

Fig. 2. Test for block of nerve impulses during responses to d.c. of relatively high voltage. Decapitate cat. The current (8 v.) was applied to electrodes placed low in the thigh with an interelectrodal distance of 8 mm., first with an ascending and later with a descending direction (pairs of signals, bottom line). Condenser shocks of an intensity 5 times maximal were delivered regularly, as shown by the twitches, to another pair of electrodes near the cut end of the nerve at the hip.

slightly greater than threshold are as a rule not single, but repetitive. Second, the effects attributed to strong currents are different from those reported by Pflüger. A probable explanation of this discrepancy, apart from the fact that Pflüger's statements refer to frog's nerves, may be that he worked exclusively with excised, that is non-circulated, nerves, whereas the present data apply to circulated nerves, in normal condition. In the excised nerves studied here the classical effects were the rule—that is, abundant repetitive discharges to the make or break of strong currents, according to whether the cathode or the anode of the d.c. was placed toward

the recording electrodes. That this difference in responses was due to the exposure and lack of circulation and not to the nature or mode of application of the electrodes was shown by the two following observations. In some cases the cut end of the nerve was freed up to one of the blood vessels supplying the sciatic, and electrodes shielded by glass tubing, of the Sherrington type, were applied to the dissected region. The results were similar to those in table 1, although it was necessary to employ higher voltages than usual. In other experiments the blood supply to the nerve was destroyed by crushing or tying all the visible vessels. The nerve was then left in its normal position and electrodes were applied to it as usually. The results were similar to those seen in the excised nerves—that is, similar to those described by Pflüger.

B. *Responses to stimulation through electrodes, one localized and the other diffuse.* In these experiments several silver chlorided electrodes were placed on the popliteal nerve, as usual, but the dry cotton pledgets employed in other cases to isolate the nerve as much as possible from surrounding tissues were avoided. A heavy chlorided silver needle was further inserted subcutaneously or intramuscularly in the middle of the thigh. The d.c. was then delivered, using this needle as one of the electrodes—the diffuse one with respect to the nerve—and any of the wires in close contact with the nerve as a localized electrode where the current density was heaviest.

TABLE 1

Muscular responses to stimulation of circulated motor nerves by direct currents through two localized electrodes

CURRENT INTENSITY	ASCENDING DIRECTION		DESCENDING DIRECTION	
	Closure	Opening	Closure	Opening
Weak	Twitch		Twitch	
Moderate	Medium tetanus	Twitch	Medium tetanus	Twitch
Strong	Small tetanus	Medium tetanus	Large tetanus	Small tetanus
Very strong	Large tetanus	Small tetanus	Small tetanus	Large tetanus

The tests were made as in the previous section, by applying d.c. of various intensities for 1 to 10 seconds, making the localized electrode alternatively anode or cathode. With progressively increasing voltages the first response detected, a twitch, corresponded invariably to the make of the current when the localized electrode was the cathode. This response grew thereafter, promptly becoming tetanic in character, and only decreased later, with fairly strong voltages (15 to 30 times rheobase).

The next response to appear was also at the closure of the current, when

the anode was localized. This response in turn increased with greater voltages, and kept on augmenting when the response to the make with a localized cathode had already begun to decrease. It was always possible to evoke with a sufficiently high voltage a greater response to the make of the current with a localized anode than with a localized cathode (figs. 3 and 4).

The responses to the break of the currents appeared exceptionally with weaker voltages when the anode was the localized electrode. The converse was, however, usually seen. The response to the break with a localized

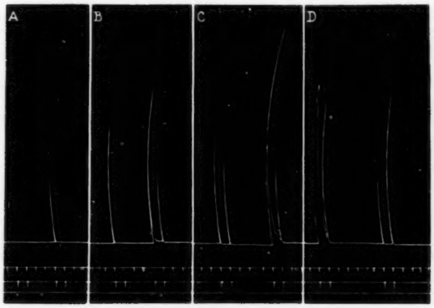

Fig. 3

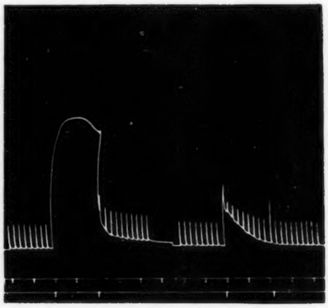

Fig. 4

Fig. 3. Muscular responses to stimulation of the popliteal nerve with electrodes, one localized (needle in contact with the nerve) and the other diffuse (needle in the subcutaneous tissue). Cat under dial. As in figure 1, the responses are arranged in pairs, the currents having been delivered with the localized electrode first as the anode and then as the cathode. The voltages for the successive pairs were: A, 1; B, 2; C, 4; D, 8.

Fig. 4. Test for block of nerve impulses during responses to d.c. of relatively high voltage. Decapitate cat. The current (10 v.) was applied through one localized electrode placed low in the thigh and another diffuse electrode as in figure 3. The localized electrode was first the anode and then the cathode. Supramaximal condenser discharges were delivered regularly, as shown by the twitches, through another pair of electrodes near the hip.

cathode could be present with currents much weaker (up to $\frac{1}{10}$) than were necessary when the anode was the localized electrode (table 2, fig. 3).

With strong currents the contractions were typically as follows. If the cathode was the localized electrode the response to the make was small, that to the break large. Conversely, with a localized anode the response to the make was large, that to the break relatively small (figs. 3 and 4).

Tests for block during or after the applications of d.c. were made by stimulating with condenser discharges of an intensity 5 to 10 times maximal a region of the nerve 3 to 6 cm. central to the point where the localized electrode was placed. The intensities of d.c. required to produce block

were greater than those which had resulted in a relative decrease of the responses to the make with a localized cathode (fig. 4). In figure 4 the localized anode elicited a practically maximal tetanic contraction. It is not possible to judge, therefore, whether or not block was present in that observation. Experiments in which the tetanic responses to d.c. were less marked than those in figure 4, however, showed that a localized cathode produced invariably block at lower voltages than did a localized anode (cf. Bishop and Erlanger, 1926).

In a few experiments in which the diffuse electrode was obtained by connecting together several electrodes placed on the nerve either centrally, or peripherally, or both, with respect to the localized single electrode, the results were quite similar to those seen with the needle in the subcutaneous tissues.

C. *Responses to brief d.c. pulses delivered repeatedly at short intervals.* As explained under Method, such brief (5.6 to 16.7 msec.) pulses were

TABLE 2

Muscular responses to stimulation of circulated motor nerves by direct currents through electrodes making one a localized and the other a diffuse contact

CURRENT INTENSITY	LOCALIZED CATHODE		LOCALIZED ANODE	
	Closure	Opening	Closure	Opening
Weak	Twitch			
Moderate	Small tetanus		Twitch	
Strong	Large tetanus	Twitch	Small tetanus	
Very strong	Small tetanus	Large tetanus	Large tetanus	Small tetanus

obtained by the regular illumination of a photoelectric cell at the rate of 30 per second. The observations were all made on excised nerves, the spike potentials of the A fibers serving as indicators of nerve activity.

In a series of observations the stimuli were delivered to one end of the nerve and the responses were recorded monophasically from the other end. The quite regular responses which resulted from stimulation by the d.c. pulses at various intensities were photographed with exposures of $\frac{1}{5}$ second during the course of stimulation. The regularity of the responses is emphasized by the fact that, although several sweeps (5 or 6) of the spot traveled over the face of the cathode-ray oscillograph during the exposures, the lines appear single on the records (figs. 5, 11 and 12).

Certain features of the responses obtained in these conditions will be described below in connection with the repetitiveness of nerves stimulated by d.c. For the present, the following important characteristics will be emphasized. With the stimulating electrodes placed one on a crushed and the other on an intact region of the nerve, the latency of the responses

to the make or break of the d.c. pulses could be made approximately the same, by proper adjustment of the voltage, whether the anode or the cathode was on the undamaged nerve. This was true even when the distance between the two stimulating electrodes was relatively long, e.g., 7.5 cm.

When the stimulating electrodes were both on intact regions of the nerve the response to the break of the d.c. pulses was often double—i.e., two submaximal volleys with different latencies. The magnitude of the two components changed independently when the intensity of the stimuli was varied. The phenomenon is illustrated in figure 5.

In another series of experiments the record was obtained from a region of the nerve placed between the stimulating electrodes. The purpose of these observations was to determine by the polarity of the diphasic

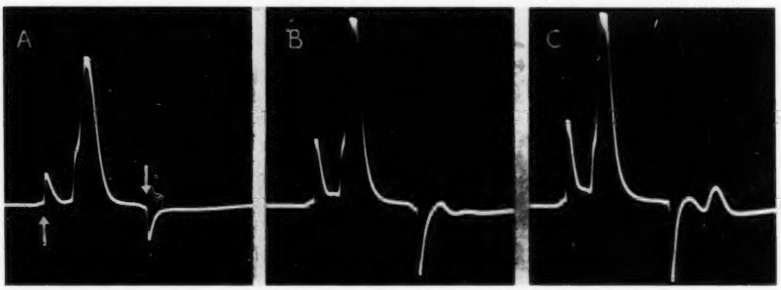

Fig. 5. Double responses to the break of d.c. pulses (5.6 msec.). The record was taken monophasically from the peripheral end of the peroneal nerve. The stimulating electrodes were placed, the anode 6.6 and the cathode 7.6 cm. away from the recording leads. Both stimulating electrodes were on intact regions of the nerve. In A no response to the break of the pulse is seen (the arrows indicate the artifacts of the make and break of the pulses). In B a response with a short latency is visible. In C an additional response appears with longer latency. Voltages (conventional units): A, 4; B, 5; C, 5.5.

response thus obtained the direction from which the nerve impulse reached the recording electrodes—i.e., the pole of the stimulus at or near which the corresponding impulses originated. For if an impulse conducted from *b* toward *c* in figure 6 should appear in the record as an "up"—then "down" excursion, an impulse traveling from *c* to *b* would record as a "down"-"up" diphasic complex.

Certain precautions are necessary for these records. The main technical problem encountered was to reduce the large d.c. artifact to sufficiently small or brief dimensions so that the response would be clearly recognizable and its polarity identified. The set-up shown in figure 6 was found satisfactory. The small capacities (C_2) placed beyond the recording electrodes effectively reduced the d.c. artifact. They further served the

purpose of eliminating a spurious anode or cathode at the points *c* and *d*, since the d.c. could not flow through such capacities. The time constant corresponding to such capacities and to the 50,000 ω potentiometer from which the Wagner ground was led, was adequate for the recording of the spike potentials without serious distortion. The Wagner ground, with or without capacity and resistance, further reduced the artifacts and was of great value, when shifted during a response, for recognizing a doubtful polarity. As may be readily understood, the results were not always clear. For if impulses should arrive at the recording electrodes from both ends of the nerve simultaneously or at close intervals the responses might collide if coming over the same fibers, or they might at least partially cancel for recording purposes even when coming over different fibers of the nerve.

Certain clear effects were encountered, however, as follows. In figure 7 are illustrated records of a typical experiment from a fresh (recently excised) nerve from a decapitate (unanesthetized) preparation. The stimulating electrodes were placed one on an intact region 3 cm. away from the peripheral cut of the peroneal nerve and the other on the crushed central end. The electrode on the intact region was first made the anode for the brief (5.6 msec.) d.c. pulses. As the voltage was progressively increased the responses to the break of the pulses appeared with the polarity corresponding to impulses set up from the region of the nerve where the anode lay. Stronger pulses led to the appearance of a response to the make of the pulses, and their polarity was similar to that of the responses to the break—i.e., they originated from the region of the nerve where the anode was placed, not from that to which the cathode was applied.

When the polarity of the d.c. pulses was reversed, that is, when the anode was in contact with a crushed and the cathode with an intact region of nerve, the first responses to appear were to the make of the current, and their polarity indicated that they came from the region of the cathode. Further intensification of the current led to the appearance of a response to the break of the pulses. This response had again the polarity corresponding to origin at the cathode, not the anode of the stimuli.

As already emphasized, this effect was commonly encountered in the fresh nerves from spinal animals. It was only rarely seen in nerves excised from animals anesthetized with dial, or in nerves which had been excised and observed for 1 to 3 hours.

Another characteristic effect seen in about one-half of the nerves studied was the following. With the stimulating electrodes placed as before, one on intact, the other on crushed nerve, a response to the make or break of the d.c. pulses appeared with a given intensity of current. Further intensification of the stimuli led then to the appearance of an additional response which preceded or succeeded the first one recorded. The polarity

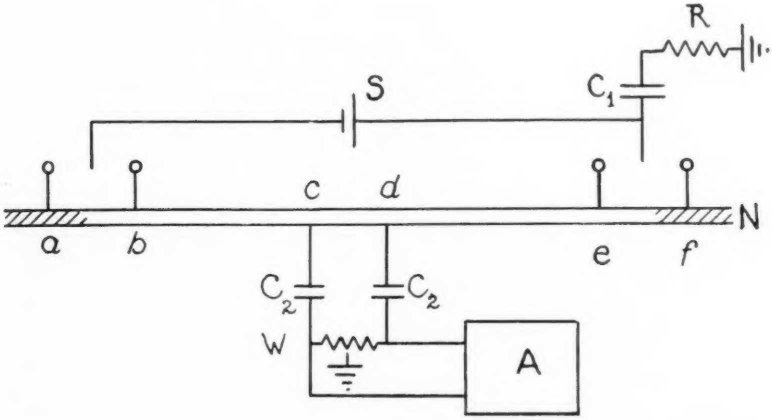

Fig. 6. Diagram of the arrangement used for recording the nerve action potential diphasically between the two d.c. poles. The letters mean: S, d.c. stimulator; R, 10,000 to 100,000ω; C_1, 0.1 μF; N, nerve, the shaded areas represent the crushed ends; *a* to *f*, electrodes; C_2, 0.001 μF; W, Wagner balance to ground through a 50,000ω potentiometer; A, amplifier.

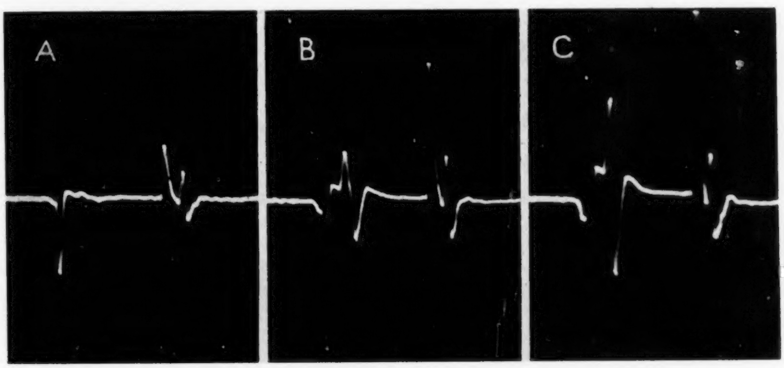

Fig. 7. Responses from the anode at the closure and opening of d.c. Excised peroneal from a spinal animal. The experimental set-up was as shown in figure 6, with the anode of the d.c. at *b* (intact nerve) and the cathode at *f* (crushed nerve). The monophasic artifacts show the beginning and end of the pulses (5.6 msec.) delivered 30 times per sec. In A only a response to the opening of the current is present; the polarity is that which corresponded to impulses traveling from *b* to *f*. In B a response to the make of the pulses appears; its polarity is similar to that of the response to the break of the pulses (as in A). In C, with a higher voltage, the response to the make is greatly increased. Voltages (in conventional units): A, 3; B, 4; C, 5.

of this additional response was opposite to that of the original one, thus showing that at either the make or the break, or both, nerve impulses were being originated, probably over different fibers, from both poles of the stimuli. Typical instances of this effect are illustrated in figure 8.

In all the observations described in this section the responses attributed to one of the poles showed a decrease or an increase of latency when the recording electrodes were moved closer to or further from that pole. In

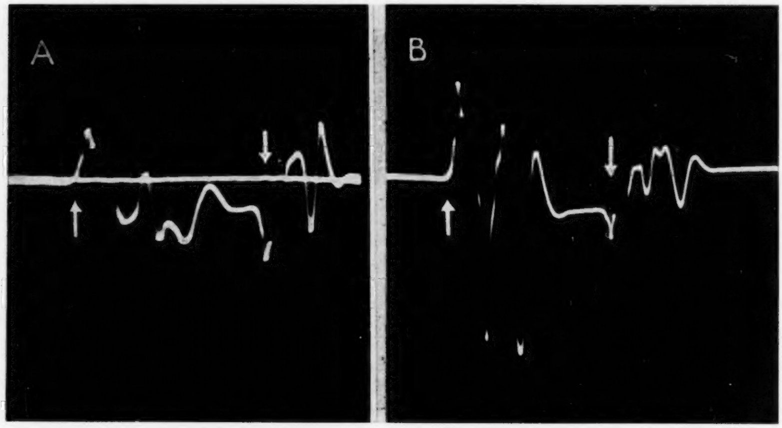

Fig. 8. Double responses of opposite polarity to make and break of the d.c. pulses (5.6 msec.). The experimental set-up was as shown in figure 6. Peroneal nerve. The arrows indicate the make and break artifacts.

In A the cathode was at *a* (crushed nerve) and the anode at *e* (intact nerve). The single response to the break of the pulses shows the polarity of the record of impulses traveling from *e* to *a*. The response to the make is double; a submaximal volley going from *a* toward *e* is followed by a volley traveling in the opposite direction.

In B the d.c. électrodes were as in A, with the anode on an intact and the cathode on a crushed region of the nerve. The recording electrodes *c* and *d* were closer to the anode than to the cathode of the d.c. pulses. The responses to both the make and the break are double; a volley from the anode is followed by a volley from some point near the cathode.

the cases of double response of opposite polarity (fig. 8) it was sometimes possible by continuous application of d.c. from another source near one of the stimulating poles to increase or decrease selectively one or the other of the two diphasic components of the complex response. A similar relative separation of these components was also obtainable by sending through the stimulating electrodes d.c. in either direction in the intervals between the stimulating pulses. As explained under Method, such additional currents could be readily obtained by changing the balance of the amplifier in the output of the photoelectric cell.

D. *The repetitive nature of the responses of nerve to continuous applications of d.c.* As already emphasized (pp. 102, 104, figs. 1 and 3), the muscular responses to d.c. were invariably tetanic when the intensity was 1.5 to 3 times that of the threshold of the most excitable motor fibers in the nerve. Slightly more intense currents led usually to the appearance of large tetani which could be well sustained for periods of over 10 seconds (fig. 4).

The conditions which favored the appearance of well-sustained repetitive responses may be summarized by the general statement that the more fresh and normal the nerve the greater the ease with which such responses were elicited. Thus, in all the experiments made on spinal animals after the ether given during anesthesia had been eliminated, well-sustained tetani could be readily elicited with weak currents. In many of the animals studied under dial anesthesia, on the other hand, although tetanic responses could always be evoked, they were only poorly sustained, even at relatively high voltages.

The presence or absence of the circulation in the nerves was also found significant. Well circulated nerves were more apt to show repetitive discharges than were nerves whose circulation had been deliberately or unwittingly damaged. Similarly, nerves *in situ* showed invariably richer repetition than did the same nerves or others from the same animals when observed even immediately after excision.

Regions of a given nerve which had been stimulated for some time yielded less repetition, even after prolonged rest, than that resulting from similar currents applied to other relatively fresh regions of the same nerve. Finally, in the excised nerves the probability of eliciting repetitive responses decreased progressively with time.

The repetitive nature of the responses to d.c. was seen regularly not only upon the closure of the currents, but also when currents of sufficient intensity were opened. Higher voltages were necessary, however, for the appearance of opening tetani than those sufficient for the production of the closing tetani. Stimulation by prolonged applications of d.c. to nerves *in situ* or excised resulted usually in quite irregular electrograms (fig. 9). Not infrequently, however, the responses of the different fibers tended to synchronize, causing then the appearance of fairly regular waves in the electrical records. In figure 10 is illustrated one of the outstanding instances of such synchronization. The records were taken at 0.5-second intervals.

The method of stimulation and recording described on p. 106 was found very satisfactory for the detailed study of the repetitive responses both to the make and the break of the current. The photoelectric cell was illuminated 30 times per second for 11.1 or 16.7 msec., the intervals between the stimuli being, therefore, 22.2 and 16.7 msec., respectively. Whether both or only one of the stimulating electrodes was in contact with intact nerve, and whatever the polarity of the stimuli with respect to the

recording electrodes, it was always possible to record repetitive responses both to the on and the off of the stimuli if the voltage was raised sufficiently.

Two interesting features were regularly seen. First, the frequency of the repetitive discharges was directly related to the intensity of the pulses delivered. Thus, by progressively increasing the voltage during a period of stimulation, when threshold was attained a response to the closure appeared. Slight intensification resulted in the growth of this first response and in the appearance of a second response late during the period of passage of the current. This second response then increased in magnitude and decreased in latency until a third response appeared. A similar process could recur until it was possible to see a regular pattern of as many as 7 waves occurring during the 16.7 msec. that the current was applied. A series of pictures illustrating the phenomenon is shown in figure 11. These pictures give only a suggestion of the thoroughly continuous and quite systematic changes seen during the gradual increase or decrease of intensity.

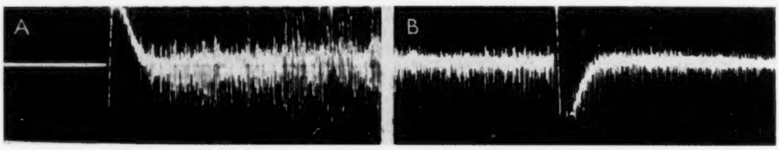

Fig. 9. Irregular responses of nerve to a prolonged application of d.c. Excised peroneal. Cathode of d.c. toward recording leads. A, closure of d.c., 2 v. B, opening of d.c., after 5-sec. application.

The responses to the break of the pulses showed likewise characteristic repetition, with frequency again proportional to intensity (fig. 12). Higher voltages of stimulation were necessary to see this effect than were sufficient to elicit marked repetition during the period of passage of the pulses. The greatest frequency recorded (7 waves during 22 msec.) was lower than those which could be readily obtained during the "on" period.

The second feature of the repetitive effects worthy of emphasis was the regularity of the responses registered. In some instances the patterns of repetition were quite complex, due probably to the fact that different groups of fibers were discharging at different frequencies and due probably, also, to simultaneous stimulation at the cathode and the anode. Yet such complex patterns could be sustained so well that pictures taken up to 1 minute apart showed only insignificant differences (cf. fig. 12, F and G).

Discussion. The classical concept, that stimulation occurs only at the cathode upon closing and only at the anode upon opening a d.c. applied to nerve, fails to account for some of the data described in sections A and B. Thus, the increase of the closing tetanus for the ascending current,

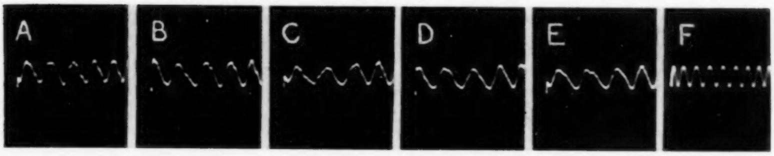

Fig. 10 Synchronized repetitive responses of nerve to a prolonged application of d.c. Excised peroneal nerve from a spinal cat. Cathode of d.c. toward the recording leads. The successive pictures, A to E, were taken at half-second intervals beginning immediately after the application of d.c. The amplitude of the synchronized waves is about 20 per cent of a maximal A spike. F, 1,000 cycles.

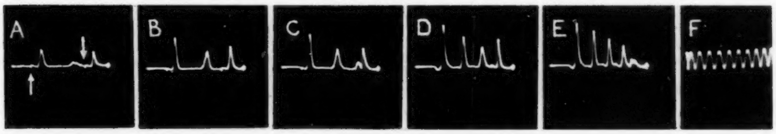

Fig. 11. Repetitive responses of nerve during the passage of d.c. Brief (11.1 msec.) pulses applied at the rate of 30 per sec. to an excised phrenic nerve from a spinal cat. The on and off artifacts of the pulses appear as small diphasic excursions (arrows in A). The cathode of the stimuli was proximal to the recording leads. The conduction distance was 6 cm. Voltages (in conventional units): A, 5; B, 6; C, 7; D, 8; E, 9. F, 500 cycles.

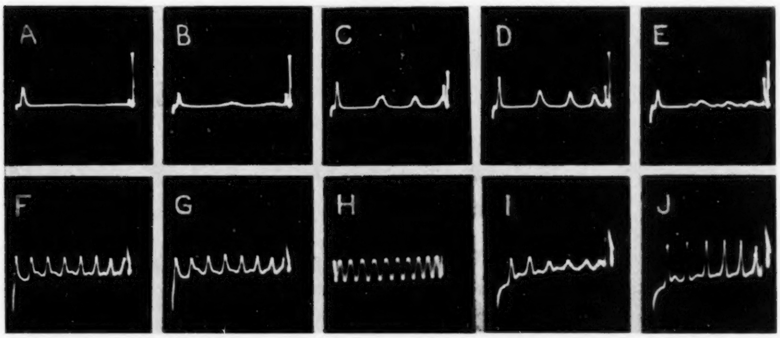

Fig. 12. Repetitive responses of nerve after the passage of d.c. Brief (11.1 msec.) pulses applied as in figure 6. The records begin with the artifact showing the break of the pulses and end with the artifact for the make of the pulses and the corresponding responses (crowded together at the end of the sweep). In records A to G the anode of the stimuli was proximal to the recording leads; in records I and J the polarity of the stimuli was reversed. Voltages (in conventional units): A, 6; B, 7; C, 8; D, 10; E, 12; F and G, 25; I, 22; J, 35. Record G was taken 1 min. after F, the nerve being stimulated continuously at a constant voltage. H, 500 cycles.

with a corresponding decrease of the response to the closure of the descending current as the voltage is intensified (fig. 1) is not in accord with that concept. The opposite effect seen in similar conditions for the responses to the opening of the currents (fig. 1) is likewise not explainable on that basis.

Pflüger (1859) attributed the difference of effects of strong ascending or descending currents to a block of the impulses set up at the pole distal to the muscle by the deleterious blocking effects of the proximal pole. It is interesting to note, however, that he did not test experimentally for this block. When such tests were made in the present study it was frequently found (fig. 2) that block of impulses set up centrally to the d.c. electrodes was negligible or absent.

The failure of a strong descending current to cause as large a muscular response as that elicited by the same, but ascending current (fig. 2) might be considered as due to Wedensky inhibition, because of too high a frequency of impulses reaching the muscle when the cathode is proximal. That this is not the case was shown by the fact that single maximal twitches could be elicited during the period of application of the current. Indeed, that the muscle was in all instances a faithful indicator of activity in the nerves was shown by the close parallelism found when the mechanograms were compared to the electrograms recorded from a region of the nerve between the d.c. electrodes and the muscle (p. 103).

From the responses in figure 2, it is clear, therefore, that the large tetanus produced by the closure of the ascending current is not due to impulses starting at the cathode. If such were the case the descending current should produce as large or a greater effect, but it does not. The conclusion appears reasonable that the impulses originate at the anode and that they are conducted in one direction only, away from the d.c. poles. An apparent paradox is thus revealed, in that whereas these impulses would be blocked toward the cathode, impulses coming from higher regions of the nerve are not blocked. The discrepancy may be solved in one of two ways. First, the observations of Hodgkin (1938) suggest that nerve impulses do not start directly as consequences of electric stimuli, but that they are preceded by a local, unpropagated response which, if sufficiently great, initiates the conducted disturbance. It is conceivable, therefore, that in figure 2 the anode sets up local responses which succeed in provoking the appearance of conducted waves only in the direction opposite the cathode. The full-sized nerve impulses reaching the anode would, on the other hand, be conducted through without block.

A second explanation could be that there is a polarity in motor nerves such that the direction of the d.c. is significant not only with respect to the muscle, but also in relation to the stimulating ability of the current. In other words, it is possible that a strong ascending current is a better stim-

ulus for a motor nerve than a descending current. This possibility is, however, rendered unlikely by the absence of any evidence of polarity in nerve fibers when the influence of d.c. on their electrical excitability is analyzed (Rosenblueth, 1941).

With the problem of one-way stimulation dismissed, the data in figure 1 and in table 1 suggest that both the anode and the cathode can stimulate both at the make and at the break of d.c. The make of the cathode appears to increase in efficiency with increasing voltage up to an optimum and then to decrease for stronger voltages. The make of the anode does not show this decrease with strong currents. The break of the anode seems to behave like the make of the cathode, while the break of the cathode behaves like the make of the anode, as the intensity of the currents is increased.

Results obtained by "unipolar" methods of stimulation were declared by Biedermann (1895) unsuitable for accurate interpretation. He pointed out that the term was inadequate; if there is a cathode in a nerve there must also be an anode, and *vice versa.* In recognition of this legitimate criticism the term unipolar has been avoided in this report and the expression localized, as opposed to diffuse, has been substituted. Biedermann concluded that it is impossible to estimate the rôle of uncontrolled spurious poles, that is, unknown regions where the current enters or leaves the nerves.

The possibility of spurious poles should, of course, be kept in mind in evaluating the experiments described in section B. Thus, the responses to the closure of weak currents with a localized anode (fig. 3B) might be attributed to spurious cathodes. With the experimental conditions adopted, however, this assumption appears unreasonable. The cathode was spread by the tissues to a quite large surface of the nerve. The points where the current would leave the nerve with greater density should be those in the close vicinity of the localized anode, that is, those where, according to classical theory, the electrical excitability should be maximally depressed. It is probable, therefore, that these responses were due to the anode and not to a hypothetical concentrated cathode.

The fact that the responses to the opening of the currents occurred usually with lower voltages when the cathode rather than the anode was localized (fig. 3) is incompatible with the classical theory. A break response, with a localized cathode, would be due to a spurious anode. Why, then, should not the certainly more concentrated effect of the localized anode yield an even greater response? On the other hand, all the results in section B (figs. 3 and 4, and table 2) fit the theory expressed above as suggested by the analysis of the results in section A.

Several authors (see Mares, 1913; Thörner, 1923; Woronzow, 1924) have come previously to the conclusion that the classical theory of nerve

stimulation by d.c. is incomplete. Their results will not be discussed here. Suffice it to say that in their experiments, like in those in sections A and B, the indicator of nerve activation was the muscular mechanical response. It might be objected that such observations furnish only indirect evidence with regard to problems of nerve function. That objection does not apply, however, to the results in section C.

Inferences on the stimulating action of d.c. based on measurements of latency are complicated by the influence of d.c. on conduction velocity. Because of this complication the latency of a response was used only sparingly to determine its site of origin in the nerve (p. 107). The polarity of a diphasic record, on the other hand, is a certain indicator of the region of the nerve from which an impulse reaches the recording electrodes. The observations in figures 7 and 8 prove, therefore, that both the anode and the cathode may stimulate nerves both at the closure and at the opening of d.c. The records in figure 8 show further that it is possible to obtain simultaneously anodal and cathodal excitation at either the make or the break of d.c. This explanation accounts satisfactorily for the complex responses to the break of the d.c. pulses in figure 5. The two components of these responses correspond to impulses set up by the anode and cathode, respectively, over different fibers.

The problem of the repetitiveness of nerve responses to continuous stimulation by d.c. has been only little studied thus far. Indeed, although, as already mentioned, Pflüger (1859) emphasized that tetani, instead of twitches, were the usual muscular responses in fresh preparations, and although this fact has been abundantly confirmed since (see figs. 1 to 4 and 9 to 12), text-books still describe twitches as the normal responses to d.c. and only mention tetani as abnormal effects of very strong currents.

In experiments on the excised dog's phrenic nerve Erlanger and Blair (1936) found intense spontaneous activity. Both single shocks and rectangular currents elicited repetitive discharges when applied to a region of the nerve with the sheath intact. If the sheath was removed the repetitive responses disappeared. These results differ from the present observations. The nerves studied showed little or no spontaneous activity, except when deteriorated by exposure or experimental handling. Repetitive responses could invariably be elicited by d.c., although the sheath (only slight in the cat as compared to the dog) was always removed as much as possible during dissection. The main difference in the experimental conditions appears to be that Erlanger and Blair observed the phrenic nerves only after they had been placed in Locke's solution in a refrigerator for several hours, whereas in these experiments the observations were made as soon as possible after excision.

Erlanger and Blair (loc. cit.) found that anodal polarization favors the appearance of repetitive responses. On this basis they suggest that the

repetition which has been observed in intact human nerves (Ebbecke, 1924) may be due to a normal state of anodal polarization descending to the nerves from the centers (subordination; Lapicque, 1923). Such an explanation cannot apply to the present results. Repetition always occurred after the nerves were cut. Indeed, in a few observations made to test the point, no differences in the muscular responses to d.c. were seen in records taken before and after cutting the sciatic nerve.

The results illustrated in figures 9 to 12 lead to the following conclusions. Repetitive discharges are the normal response of nerve fibers to continuous applications of d.c. of voltage slightly greater than threshold. The fresher and more normal the nerve, the greater its ability to exhibit repetition (p. 111). Although the rates at which individual fibers repeat in response to a given treatment with d.c. may vary considerably, the integrated effect on a large number of fibers shows statistical uniformities—i.e., a large majority of the A fibers in a nerve tend to repeat at the same rate, especially if stimulated by brief d.c. pulses. The number of fibers sharing in the successive responses during repetition and the frequency of the repetition increase with the voltage of the d.c. applied. The preceding statements apply both to the closure and to the opening of d.c.

The changes of the electrical excitability of nerve produced by applications of d.c. are described and discussed in a separate report (Rosenblueth, 1941). It may be mentioned here that those changes are in agreement with the conclusions inferred in the present study. Thus, if it is possible to obtain stimulation by the make at the anode and by the break at the cathode, it should be possible to demonstrate increases of excitability in these conditions with subthreshold currents. This was found to be the case.

It is hardly necessary to point out that the results reported in this study and the inferences derived therefrom entail a serious revision of current theories of nerve function. These theories are based on simplified premises drawn from the classical experiments, made almost exclusively on excised frogs' nerves. For example, as mentioned before, although it has been repeatedly shown that the common response of normal nerves to d.c. is not single but repetitive, yet emphasis is usually placed on the marked ability of nerve to "accommodate" to a continuous stimulus. Similarly, although Pflüger (1859) reported that the most prominent aftereffect of cathodal polarization is frequently an increased, instead of a decreased excitability, only the post-cathodal depression has been incorporated in the current concepts. The study of circulated or freshly excised mammalian nerves confirms all the positive classical findings and brings out additional data. It is likely that these new properties will also be exhibited by batrachian nerves. But even if such should not be the case a general theory of nerve should include all the data.

SUMMARY

The responses of circulated cat's motor nerves to ascending or descending direct currents (d.c.) of variable voltage (figs. 1 and 2; table 1) suggest that stimulation may occur not only at the cathode at make and at the anode at break, but also in the reversed relation.

The suggestion is supported by the results of stimulating through a localized electrode on the nerve and a diffuse lead through the surrounding tissues (figs. 3 and 4; table 2).

Observations made on excised nerves (figs. 5 to 8) give direct evidence that the suggestion is correct.

Applications of prolonged d.c. or of brief pulses result in repetitive responses from the nerves if the voltage is over 1.5 to 3 times rheobase (figs. 1 to 4 and 9 to 12). The frequency of this repetition increases with the voltage of the d.c. (figs. 11 and 12). These statements apply both to the closure and the opening of d.c., but repetition at the opening requires higher voltages than repetition during the passage of the current.

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THE STIMULATION OF MYELINATED AXONS BY NERVE IMPULSES IN ADJACENT MYELINATED AXONS

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The stimulation of nerve fibers by the activity of adjacent muscle elements is well known. The activation of non-myelinated nerve fibers by nerve impulses traveling over similar adjacent elements was occasionally seen by Jasper and Monnier (1938). Arvanitaki (1940) treated the region where two such fibers were in contact with sodium citrate, in order to increase the local excitability. In those conditions a nerve impulse in one of the fibers elicited regularly an impulse in the adjacent element, with a latency of less than 5 msec.

No observations of a similar phenomenon have been reported for myelinated fibers. Indeed, Blair and Erlanger (1932), on the basis of negative results, concluded that the nerve impulses in a myelinated fiber fail to produce even a subliminal excitation of the adjacent elements.

The present report describes conditions in which activation of myelinated mammalian axons will regularly occur when nerve impulses travel through the neighboring elements in the same nerve trunk.

METHOD. The nerves studied were mainly the peroneal and occasionally the popliteal and phrenic of the cat. The animals were either anesthetized with dial (Ciba, 0.75 cc. per kgm.) or decapitated under ether anesthesia. In the decapitate animals artificial respiration was administered and the anesthesia discontinued. One-half to four hours later the nerves were dissected and excised. They were then placed in a moist chamber for study.

The nerve action potentials were recorded monophasically or diphasically from a cathode-ray oscillograph, after adequate amplification.

The test stimuli were condenser discharges through a thyratron, rendered diphasic by means of a transformer. They were delivered at frequencies of 2 to 60 per sec. Direct currents (d.c.) were sent to the nerves through impolarizable calomel electrodes.

In some experiments the contractions of the tibialis anticus muscle were used as indicators of nerve impulses in the motor fibers of the peroneal nerve, cut centrally. The leg was then fixed by drills inserted into the tibia. The tendon of the muscle was attached to a tension myograph. Upward excursions in the kymograph records denote contraction.

RESULTS. Three pairs of electrodes were placed on the excised nerves, one for recording purposes, one for the test stimuli, and one for d.c. For convenience in referring to the position of the electrodes the following convention will be used: r_1 and r_2 will indicate the recording pair, s_1 and s_2 the pair used for the test stimuli, and c_1 and c_2 the pair of impolarizable electrodes used for d.c.

In a series of observations r_1 and r_2 were placed toward either end of the nerve, r_1 being on the crushed extremity. Next came s_1 and s_2; and then c_1 and c_2, both pairs on intact regions of nerve. In these conditions, if the stimuli were submaximal, a simple A spike was present in the records. Applications of d.c. through c_1 and c_2 resulted, of course, in changes in the amplitude of the original spike, due to changes of excitability and of the spike magnitude of the responding elements. But in addition to these

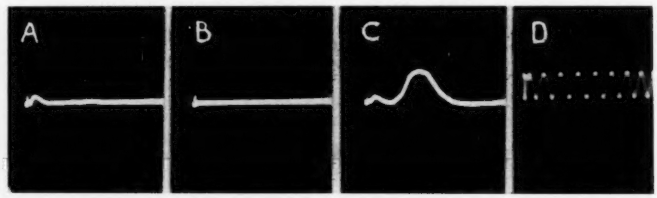

Fig. 1. Appearance of a late response after application of d.c. Monophasic record from a region 1.5 cm. away from the crushed central end of an excised peroneal nerve. Stimulating electrodes: anode 2.2 and cathode 3.0 cm.; and d.c. electrodes: anode 5.3 and cathode 7.0 cm. away from the same end of the nerve. Submaximal stimuli applied regularly at ½-sec. intervals.

A. Response before application of d.c. B. Absence of response during the passage of d.c. (4 v.). C. Early and delayed responses 1 sec. after opening of the d.c. D. One thousand cycles.

well-known changes, the electrogram was frequently complicated by the appearance during or after the passage of d.c. of 1 or 2 later spike potential waves (figs. 1 to 5).

These late waves were spread out in time, that is, they showed considerably more temporal dispersion than the first early response. As a rule, the late spikes were smaller than the early response (figs. 2 and 3), but occasionally the amplitude of one or both of them could be larger than that of the first spike (figs. 1 and 4). If the intensity of the stimuli was too small (activation of only a few fibers) or too great (maximal A initial response) no delayed spikes could be detected with any intensity or polarity of d.c.

With a fixed position of the recording and stimulating electrodes the latency of the delayed spikes varied with the position of the d.c. electrodes on the nerve as follows. With relatively weak d.c. (about twice rheobase)

there was usually only one late spike during the passage of the current. The latency of this spike depended on the position of the cathode of the d.c. The greater the distance between this cathode and the stimulating electrodes, the longer the interval between the early and the late response.

With relatively weak d.c. only one late spike appeared after the current was broken. The latency of this wave depended on the position of the

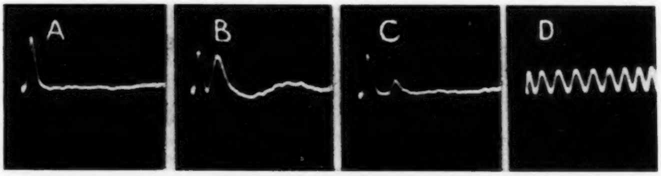

Fig. 2. Appearance of late responses during and after applications of d.c. Monophasic record from a region 2.7 cm. away from the peripheral cut end of the peroneal nerve. Stimulating electrodes: cathode 4.3 and anode 5.6 cm.; and d.c. electrodes: cathode 7.1 and anode 8.6 cm. from the same end.

A. Early response to a submaximal test stimulus. B. During the application of d.c. (0.4 v. across the nerve). The late wave starts at the cathode. C. One second after opening the d.c. The late wave starts now at the anode, hence its longer latency. D. Calibration of sweep, 1,000 cycles.

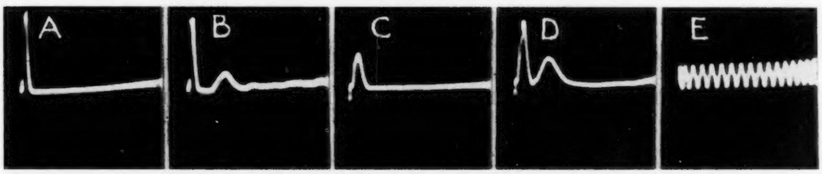

Fig. 3. Appearance of late responses after applications of d.c. Monophasic record from a region 1.5 cm. away from the central cut end of the peroneal nerve. Stimulating electrodes: cathode, 3.0 and anode 4.5 cm.; d.c. electrodes: 6 and 7.5 cm. from the same end.

A and B. During, and 2 sec. after the application of 0.2 v. d.c. with the anode distal to the recording end.

C and D. As in A and B, but with the anode of the d.c. proximal to the recording end. The latency of the late response is shorter in D than in B.

E. One thousand cycles.

anode of the d.c. (fig. 3). When with stronger currents two late waves were present the latency of each varied with the distance to the stimulating cathode of the anode and cathode of the d.c., respectively.

Changes in the interval of time separating the initial and any of the late responses could also be produced by keeping the position of the d.c. and recording electrodes constant while varying the site of application of the stimulating electrodes. The results were similar to the previous—i.e.,

with short distances between the stimulated region and the region treated by d.c., the late responses followed promptly the first spike, and conversely with long distances a marked delay separated the responses.

When the distance between the d.c. electrodes was short the delays of the late waves obtained in different conditions varied within a narrow range. When the d.c. interelectrodal distance was long some of the late responses, those attributable to the distal pole, occurred with much longer delays than those depending on the position of the proximal pole.

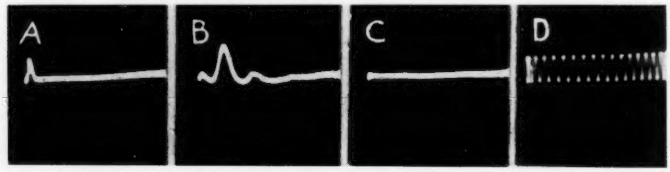

Fig. 4. Appearance of two delayed responses during the passage of d.c. Set-up as in figure 1 except that the distance between the d.c. electrodes was 1.2 cm. and the cathode, instead of the anode, was proximal to the stimulating electrodes. Voltage: 1.0.

A, before; B, during; and C, after application of d.c. D. One thousand cycles.

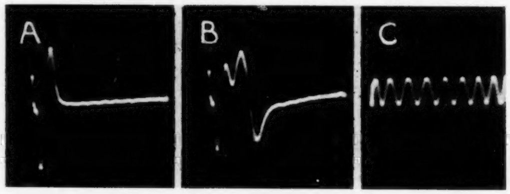

Fig. 5. Late response recorded diphasically between the d.c. and the stimulating electrodes. Excised peroneal from spinal cat. Stimulating electrodes toward the central end and d.c. electrodes toward the peripheral end of the nerve. In A is shown the submaximal early response before the application of d.c. B was taken 2 sec. after an application of d.c.: the early response is followed by a late diphasic excursion with polarity opposite to that of the first response. C. One thousand cycles.

The distance between the stimulating and the d.c. electrodes affected not only the latency, but also the magnitude of the late waves. Thus, if the d.c. and the recording leads were fixed in position, and the voltage and polarity of the d.c. was also maintained constant, shortening the interval between the stimulating cathode and the d.c. electrodes resulted in an increase of amplitude of any late waves present, while lengthening this interval caused a decrease of amplitude. In some experiments, especially if the nerves had been excised for several hours, late responses could only

be seen with relatively short distances between the d.c. and the stimulating electrodes.

If one of the d.c. electrodes was placed at the crushed end of the nerve and the other 1.5 to 3 cm. away on an intact region, only one late spike could be obtained. If the pole in contact with the intact nerve was the cathode the late spike could be readily elicited during the passage of the currents; strong currents were necessary for the additional response with the reversed polarity of the d.c. The late spike appearing at the opening of the current could likewise be seen with either polarity; it was present with lower voltages, however, if the anode, instead of the cathode, was on normal nerve.

In another series of experiments the recording electrodes were both on intact points of the nerve, so that a diphasic record ensued. The advantage of this mode of recording was that it showed, by the polarity of the response ("up-down" or "down-up" on the records) the region of the nerve from which the impulses were coming. If the nerve was stimulated toward the middle and the d.c. was applied toward one end, the record being taken from the other end, the polarity of both the early and the late spikes was the same. If, however, the recording electrodes were at the middle region of the nerve, the other two pairs being toward the ends, respectively, the polarity of the late spikes was opposite to that of the early response (fig. 5).

The conduction velocity of the late responses was measured as follows. The d.c. electrodes were placed toward one end of the nerve. The stimulating electrodes were in the close neighborhood. Records were taken during the application of d.c. with a given voltage and polarity, and with one of the recording leads at the opposite crushed end and the other lead first close to and then far from the stimulating electrodes. The difference in delay between the responses recorded in the two positions corresponded to the conduction time for the stretch of nerve lying between the two points to which the movable lead was applied. The conduction velocities of the late waves thus calculated varied between 50 and 80 m. per sec. in different observations. Specifically in a typical case the conduction velocity of the fibers involved in a given late response, over 4 cm. of nerve, was 63 m. per sec., while that of the fastest fibers contributing to the early response was 88 m. per sec.

In some observations the branches of the peroneal nerve at the lower part of the thigh were separated for about 3 cm. into two bundles. A separate pair of electrodes was applied to each of these bundles in the moist chamber. One of these pairs was used for stimulation, the other for recording. Control tests showed that such stimulation of one of the bundles, even when 4 to 6 times supramaximal, did not cause any responses (by spread) in the other bundle. When d.c. was applied to the peroneal trunk

at a more central region the just maximal or even submaximal stimuli to one of the bundles caused appreciable responses of the fibers in the other bundle. Such responses could appear either during or after the application of d.c. to the main trunk. Their latency depended on the position of the d.c. electrodes. Figure 6 illustrates an example of this type of experiment.

The experiments carried out on tibialis anticus were as follows. The muscle was hooked up for mechanical recording. A pair of chlorided silver electrodes was applied to the superficial branch of the peroneal nerve, at the ankle. Another similar pair of electrodes was applied to the peroneal trunk in the thigh; the nerve was cut 3 to 4 cm. more centrally.

The results of a typical experiment are illustrated in figure 7. Short (about 0.1 sec.) tetanic series of stimuli, with an intensity just threshold for A fibers, were delivered regularly (about 1 per sec.) to the superficial peroneal throughout the record. Those stimuli were ineffective except when d.c. (0.5 v.) was applied to the peroneal trunk, as shown by the lower signals.

Discussion. Four explanations are suggested *a priori* for the late responses in figures 1 to 5: *1*, repetitive discharges of some of the fibers yielding the early spike; *2*, the activation by the stimuli of additional, slower nerve fibers during or after the passage of d.c.; *3*, the appearance of axon reflexes in some branched fibers, if present; *4*, the activation of additional fibers by the nerve impulses set up by the stimuli when those impulses reach the regions of the nerve where the applications of d.c. have increased the local excitability.

The explanation of the late waves by repetitive discharges is rendered quite unlikely by the following considerations. The late waves may be much larger than the first response (figs. 1 and 4). Beyond a certain intensity of submaximal stimulation stronger stimuli, which activate more fibers, evoke decreased late waves (p. 120). Maximal stimulation of the A fibers fails to elicit any of the delayed spikes. If these spikes were due to repetition they would increase with the stronger stimuli. Finally, the experiments made on the split peroneal nerve (fig. 6) and on tibialis anticus (fig. 7) cannot be accounted for on the basis of repetition.

The explanation that the additional spikes might be due to the stimulation of additional slower fibers is also inacceptable. Maximal test stimuli would then evoke maximal late effects, but they do not. The delay of the late responses would depend primarily on the relative distance between the recording and the stimulating electrodes, whereas this delay was found to depend upon the relative position of the three pairs of electrodes (p. 121). The conduction velocity of the fibers contributing to the delayed spikes was found only slightly less than that of the fibers involved in the early response (p. 123). The present explanation fails also to account for the observations made on the split peroneal and on the tibialis anticus.

The third explanation, that the delayed spikes could be due to branches of some axons being activated only in the special conditions caused by d.c. is likewise improbable. In all the experiments, turning the nerve around—i.e., applying any of the electrodes to the central instead of the peripheral end of the nerves—made no difference in the results. It is therefore clear that there was no polarity in the nerves with regard to the phenomenon in question. Were branched axons significant such polarity would be expected. Again, the latency of the responses to axon reflexes would not be mainly determined by the position of the d.c. electrodes, as was found the case.

All the data are in satisfactory agreement with the explanation that the results are due to stimulation of inactive nerve fibers upon the arrival of

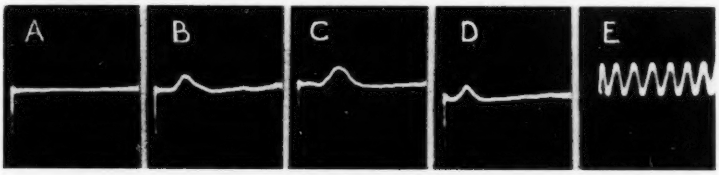

Fig. 6. Activation of adjacent nerve fibers during the passage of d.c. Peroneal nerve with the peripheral branches separated into two bundles. Test stimulating electrodes on one of these bundles and monophasic recording electrodes on the other.

A. Absence of response in the recording fibers upon supramaximal stimulation of the other bundle.

B. Similar stimulation during application of d.c. (1 v.) to the whole peroneal trunk. The cathode of the d.c. was 4 cm. and the anode 5 cm. away from the recording electrodes.

C. As in B, but polarity of the d.c. reversed. The cathode of the d.c. is now distal to the recording leads and the latency of the response is longer.

D. As in B but d.c. electrodes moved 2 cm. closer to the recording leads. The latency of the response is briefer.

E. Sweep calibration, 1,000 cycles.

nerve impulses at a region of the nerve rendered hyperexcitable by the d.c. As Pflüger (1859) showed, the excitability of nerves can be increased at the cathode during the passage of d.c., and at both the cathode and the anode after the current stops flowing. It has been shown recently (Rosenblueth, 1941) that there may also be increased excitability at the anode during the passage of d.c. The appearance of 1 or 2 late waves during or after applications of d.c. is therefore readily explained.

In the experiments illustrated in figure 5 the opposite polarity of the early and late responses is consistent with this interpretation. The first spike traveled from the stimulating electrodes at one end, whereas the late spikes were conducted away from the d.c. electrodes at the opposite end of the nerve.

The changes in latency of the delayed responses with changes in the relative position of the electrodes were precisely as the hypothesis demands. Thus, the maximal intervals between the early and the late responses were obtained with first the recording and then the stimulating electrodes near one end of the nerve, and the d.c. electrodes as far as possible toward the other end. In these conditions the direct response would be quite prompt, whereas the delayed one would involve the maximal conduction distance, first toward the d.c. electrodes and then away from them, plus the time lost in the activation of the new fibers. Conversely, if either the recording

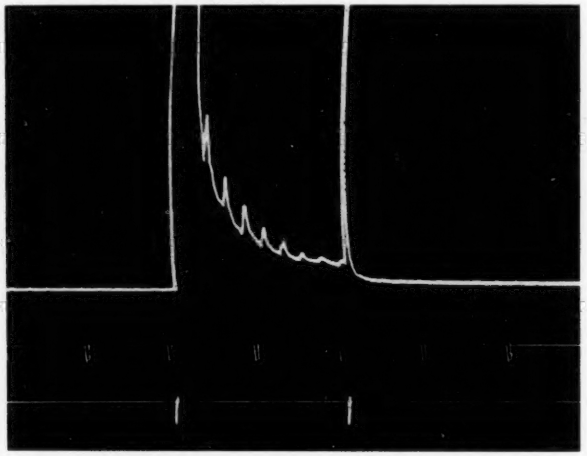

Fig. 7. Decapitate cat. Record of the contraction of tibialis anticus. Brief tetani were delivered regularly to the superficial branch of the peroneal nerve at the ankle during the record. The lower signals show the period of application of d.c. (0.5 v.) to the peroneal trunk in the thigh. The large responses to the on and the off of the d.c. were cut for reproduction, in order to emphasize the small responses to the test tetani during the period of application of d.c. Time signal: 5-sec. intervals.

or the stimulating electrodes were close to the d.c. poles, the delay of the late responses was minimal, as is readily understood.

As a final argument in support of the explanation adopted it may be pointed out that it accounts readily for the observations made on the tibialis anticus (fig. 7). The impulses traveling centripetally over the superficial branch of the peroneal nerve activate the hyperexcitable region of the peroneal trunk treated by d.c.; motor impulses are then conducted centrifugally to the tibialis and a pseudo-reflex is established. A similar explanation applies to the observations with the split peroneal nerve (fig. 6).

The conduction velocities measured in the excised nerves for both the immediate and the delayed responses (p. 123) indicate that the impulses are carried over myelinated fibers. This is obviously also the case for the experiments on the tibialis anticus. It may therefore be concluded that the myelin sheath does not provide sufficient insulation to prevent the influence of nerve impulses carried by some of the fibers on the adjacent axons in a nerve trunk.

As mentioned earlier, Blair and Erlanger (1932) obtained only negative results when attempting to demonstrate an action of nerve impulses in myelinated fibers on neighboring axons in a trunk. In some of their experiments d.c. was employed, as in the present study, to increase the excitability of the nerve at a given region. Judged from the present data, it is probable that their negative results were at least partly due to the use of weaker d.c. than necessary for the demonstration of the effect. They used subrheobasic intensities, whereas the observations reported here were all made with voltages well above threshold. It is also possible that in some of Blair and Erlanger's experiments the stimulating electrodes were too far away from the region of the nerve treated by d.c. A long distance is unfavorable to the appearance of the phenomenon in question (p. 122). The significance of this influence of distance will be discussed below.

Jasper and Monnier (1938) found delays of about 20 msec. between the arrival of the nerve impulse in one axon at the junctional region and the initiation of the response of the second fiber. Such a prolonged delay is incompatible with the hypothesis that the stimulating agent delivered by the exciting axon is its spike potential. Arvanitaki's (1940) more accurate measurements, however, show delays of 5 msec. or less. His records show further that the spike potential of the stimulated axon develops at the peak of a "local response" (Hodgkin, 1937) similar to that evoked by an electrical stimulus.

The order of magnitude of the axon-axon delay was calculated in this study by subtracting from the total delay for the arrival of the second response at a given point the time necessary for conduction of the initial and the second impulses over the corresponding nerve paths. Such calculations yielded values of not more than 1 to 2.5 msec. in several experiments. These very brief intervals are quite compatible with the hypothesis that the fibers contributing the late responses are stimulated by the spike potential of the fibers involved in the first conducted wave.

This hypothesis is further supported by the influence of the distance between the d.c. and the stimulating electrodes on the amplitude of the late responses. If the stimulus for the second response were any of the other events attending the passage of a nerve impulse (liberation of K, of acetylcholine or adrenaline, etc.) this marked influence of distance would not be readily explained. The only obvious effect on a nerve volley

of a long conduction distance is a slight temporal dispersion. This temporal dispersion will decrease the amplitude and the rate of development of the spike potential at the site of application of d.c. The important influence of amplitude and rate of growth of an electric pulse on its stimulating effectiveness needs hardly to be mentioned.

In describing their observations on juxtaposed unmyelinated axons Jasper and Monnier (1938) spoke of an "artificial synapse." Similarly, Arvanitaki (1940) mentioned a synaptic relationship involving an afferent and an effector axon. The use of such terms in this context is misleading. Transmission across an axon-axon junction and across a synapse may be proved by future experiments to follow analogous mechanisms. To assume *a priori* this similarity of transmission is unjustified. Observations on axons will throw light on problems of synaptic function if synapses turn out to be analogous to axons. Such observations, however, will not be directly relevant to the knowledge of synapses if synapses happen to be different from axons not only in structure but also in mechanism and function, as is suggested by the evidence now available.

SUMMARY

When the excitability of cat's myelinated axons is sufficiently increased at any region by applications of direct current, nerve impulses carried by some fibers stimulate the adjacent fibers (figs. 1 to 7).

The mechanism of this stimulation and the bearing of the data on the problem of synaptic transmission are discussed.

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THE INFLUENCE OF PREGNANCY AND SEX HORMONES ON GALL BLADDER MOTILITY IN THE GUINEA PIG

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Of the factors believed to play a rôle in gall stone formation, the stasis of bile has always been mentioned as of prime importance. In addition, it has long been noted that gall stones occur predominantly in women of middle age, especially in those who have borne children. A possible relationship between pregnancy and stasis of bile was thus suggested. This subject was investigated radiologically in the human by Gerdes and Boyden (1) who found that of 13 women in the 2nd and 3rd trimesters of pregnancy, all but one showed a decreased rate of emptying of the gall bladder. The mean discharge in these 13 cases, 40 minutes after the administration of the Boyden meal was only 52 per cent of the total gall bladder volume, compared with the nulligravid mean of 73 per cent. Five of these individuals were retested 6 to 8 weeks post-partum and showed marked recovery in the rate of emptying. There is no known explanation for this phenomenon and attention is naturally directed to lower animals in order to study the mechanisms involved.

Mann and Higgins (2) had previously noted that, in the guinea pig, gopher and, with some exceptions, in the dog, the gall bladder of pregnant animals usually does not empty following a fat meal, while in the non-pregnant animals it does. This placed at our disposal a suitable animal for investigation, namely, the guinea pig. In their report Mann and Higgins did not include specific data; therefore it was necessary to repeat their experiments in an attempt to confirm the statement that the gall bladder of the pregnant guinea pig does not empty as well as that of the non-pregnant pig; and further, if a difference existed, to determine it quantitatively.

Experimental. *Gall Bladder Volume of Non-pregnant and Pregnant Pigs—Unequal body weights.* The nature of some of our experiments necessitated the establishment of the normal size of the gall bladder of non-pregnant and pregnant guinea pigs. Throughout the different phases of the work, control animals were used and yielded the results in table 1 for

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normal control gall bladder volumes as determined by displacement in S-R solution.

In the non-pregnant animals body weight was found to be the only significant factor influencing gall bladder volume and the correlation coefficient was found to be + 0.470.

In the pregnant animals total body weight was also found to be the most important factor in the determination of gall bladder volume and the correlation coefficient in this instance was + 0.401. A lesser correlation (+0.368) existed between length of gestation and gall bladder size and none whatever (−0.070) between total body weight and length of gestation.

Comment. A rough correlation is thus seen to exist between total body weight and gall bladder volume in both non-pregnant and pregnant

TABLE 1

	NUMBER OF ANIMALS	MEAN G.B. VOLUME (DISP. + S.E. OF MEAN)	MEAN BODY WEIGHT
		cc.	
Non-pregnant	29*	1.33 ±0.182	602
Pregnant	19	1.90 ±0.162	844†

* Includes 20 females and 9 males.
† Weight includes the products of conception.

TABLE 2

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL.
		grams	*cc.*
Non-pregnant	15	748	1.75
Pregnant	15	766*	1.80

* Weights include products of conception.

animals, and for this reason, in all experiments in which gall bladder volume was a factor, only guinea pigs of approximately similar body weights were compared.

Equal body weights. To determine whether, on an equal weight basis, the gall bladder of the pregnant was significantly larger than that of the non-pregnant animal, we have collected and compared the gall bladder volumes of 15 non-pregnant and 15 pregnant animals whose body weights were comparable (table 2).

Comment. The data (table 2) indicate that between non-pregnant and pregnant guinea pigs of equal body weights, little or no difference exists in gall bladder volume, when the products of conception are not subtracted from the maternal body weight. Without the products of conception the

pregnant animals in table 2 would weigh less than the non-pregnant animals.

Gall Bladder Emptying in Non-pregnant and Pregnant Animals with Intact Biliary Tracts. Two types of experiments were performed: A, feeding of olive oil, and B, intravenous injection of cholecystokinin.

A. *Feeding of olive oil.* As mentioned above, Mann and Higgins have reported that the gall bladders of pregnant pigs did not empty in response to the feeding of egg yolks and cream. This type of experiment was repeated, using olive oil which in our preliminary tests seemed to produce somewhat better emptying. After a fast of approximately 24 hours, 10 cc. of fresh olive oil were fed. Four hours later the gall bladder was removed and its volume measured by displacement. The resultant gall bladder volumes of both pregnant and non-pregnant animals are shown in table 3 with a corresponding group of controls.

TABLE 3

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL. AND S.E. OF THE MEAN
		grams	*cc.*
Non-pregnant			
Unfed	29	602	1.33 ±0.182
Fed	21	555	0.89 ±0.176
Mean emptying			0.44 ±0.253*
Pregnant			
Unfed	14	745	1.68 ±0.162
Fed	20	686	1.28 ±0.189
Mean emptying			0.40 ±0.249*

* S.E. of difference of the two means.

Comment. The interpretation of the data and the differences shown is difficult. If one used only visual inspection, as did Mann and Higgins apparently, one might conclude that there was a difference between the gall bladder of the non-pregnant and pregnant guinea pigs after olive oil. This is because after feeding the average volume of the non-pregnant gall bladder was 0.89 cc. and of the pregnant, 1.28 cc., a difference of 30 per cent. But the gall bladder of the unfed pregnant animals was larger than that of the unfed non-pregnant. Calculating the percent evacuation, the non-pregnant animals emptied 33 per cent ± 17 per cent of the bile, whereas the pregnant emptied 24 per cent ± 15 per cent of the bile. The difference is not significant but the trend is toward slower evacuation in the pregnant animals. If one calculated the volume of bile evacuated, a

significant difference is not obtained, but there is again a slight trend toward less bile being emptied by the gall bladder of the pregnant animals.

B. *Intravenous injection of cholecystokinin. Method.* Under sodium pentobarbital anesthesia, the upper abdomen was opened and the incision flaps retracted until the gall bladder could easily be seen. Then, after a short recovery period, 0.5 mgm. cholecystokinin was injected into the femoral vein. The cholecystokinin used was SI, prepared in this laboratory according to the method described by Greengard and Ivy (3). (A preliminary study had indicated that all "non-pregnant" gall bladders responded to this dose of cholecystokinin.) The onset and degree of contraction were noted and 10 minutes after injection the gall bladder was removed and its volume measured by displacement. Eighteen non-pregnant, sixteen pregnant and three puerperal animals were used in this experiment.

TABLE 4

	NUMBER OF ANIMALS	MEAN BODY WEIGHT	MEAN G.B. VOL. AND S.E. OF MEAN
		grams	*cc.*
Non-pregnant			
Uninjected	29	602	1.33 ±0.182
Injected	18	642	0.69 ±0.084
Mean emptying			0.64 ±0.200*
Pregnant			
Uninjected	15	766	1.80 ±0.214
Injected	16	788	1.49 ±0.191
Mean emptying			0.31 ±0.287*

* S.E. of difference of the two means.

Results. The gall bladder sizes of both pregnant and non-pregnant pigs 10 minutes after injection of CCK are listed with a corresponding group of control animals in table 4.

In one animal in the second day of puerperium and one in the third day, there was no gall bladder emptying whatever after injection of 0.5 mgm. CCK, while in one animal in the sixth day of puerperium there was a prompt and well sustained contraction of the gall bladder.

The intravenous injection of CCK proved to be an effective and consistent stimulus to gall bladder emptying. Of the 18 non-pregnant animals, 11 showed a prompt, easily-discernible contraction of the gall bladder. In these cases white contractile areas appeared on the fundus within 15 to 60 seconds following injection. These areas became larger, coalesced and the gall bladder evacuated most of its contents within 5 to

7 minutes. In the majority of the remaining animals of the non-pregnant group, the contractions were somewhat slower in onset and seemed to produce a lesser degree of emptying. In 2 of the 18 there were no visible contractions and no apparent emptying of the gall bladder.

Of the 16 pregnant animals injected, there were no visible contractions in 8 cases; slight contractions, slow in onset and relatively ineffective in 5 cases, and strong, effective contractions in 3 cases.

Comment. The mean emptying indicated in table 4 is significant in the case of the non-pregnant animals, but not in the pregnant animals, since the gall bladders did not evacuate well in response to CCK. So, both visual and statistical evidence indicates that there is a difference in the response of the gall bladder of the pregnant and non-pregnant guinea pig to an intravenous injection of CCK. In the same

TABLE 5

PREGNANT ANIMALS	NUMBER OF ANIMALS	LENGTH OF GESTATION	MEAN WEIGHT	MEAN G.B. VOLUME
		days	*grams*	*cc.*
Uninjected*	11		696	1.84
Injected	4	15–41	665	1.08
Mean emptying				0.76
Uninjected	19		844	1.90
Injected	12	46–65	830	1.62
Mean emptying				0.28

* As mentioned previously, in the pregnant control animals, a higher correlation existed between total body weight and gall bladder volume than between length of gestation and gall bladder volume, therefore the former was used as the basis of comparison with regard to the normal volume of the pregnant guinea-pig gall bladder.

period of time (10 min.) the mean discharge of the non-pregnant gall bladder was approximately twice that of the pregnant gall bladder.

Length of Gestation and Gall-Bladder Response to CCK. The question now arises as to the influence of length of gestation on gall bladder response.

Of the 16 pregnant animals injected, there were 4 with gestation periods ranging from 15 to 41 days and in all of these there were visible contractions of the gall bladder following injection of CCK; in 3 of the 4, there was definite evidence of considerable emptying. There were 12 animals with gestation periods ranging from 46 days to term (approximately 64–68 days in the guinea pig) and of these there was visible evidence of contraction in 4 animals, and evidence of marked emptying in only 2 animals. The actual gall bladder volumes after injection of CCK in these two groups of animals, with corresponding control groups, are shown in table 5.

Comment. While the number of animals in the above groups (table 5) is relatively small, the marked difference in mean emptying of the two groups seems to indicate that the response of the gall bladder to intravenous CCK is greater in early pregnancy than in late pregnancy.

Response of Fetal Gall Bladder. It was noted that whether or not the mother had been previously injected with 0.5 mgm. CCK, all fetal gall bladders were uniformly non-contracted. In an attempt to determine if the fetal gall bladder could be made to contract by maternal injection of CCK, 2 mgm. (or 4 times the dose required to contract the non-pregnant gall bladder) were injected into the femoral vein of 5 pregnant animals and the fetuses delivered 15 to 30 minutes later. In no case was there any evidence of contraction of the fetal gall bladder.

Further injection of CCK at this time into the fetal circulation was also uniformly unsuccessful in producing contraction of the gall bladder of the fetus. However, if the newly delivered animal was kept viable for 3 to 4 hours, intravenous injection of small amounts of CCK then produced prompt contraction of the gall bladder.

Comment. The refractoriness of the gall bladder of newly-delivered fetuses makes it impossible to draw any conclusions from the above experiments with reference to the placental transmission of cholecystokinin. Two possibilities are suggested however, to explain this temporary refractoriness to CCK: first, that circulatory adjustments have to be made by the fetus to promote greater blood supply to the gall bladder and permit its contraction, and second, that a chemical or physico-chemical factor in maternal and new-born fetal blood is responsible for a partial or total inactivation of CCK.

Sex Hormones. Although the results of the feeding experiments are inconclusive, the experiments involving injection of CCK give evidence that in guinea pigs *with intact biliary tracts*, there is a decreased emptying power of the gall bladder during pregnancy and this is most marked in the latter part of the gestation period.[2] Such a difference having been established, attention is naturally directed to the cause of the difference. One of the important possibilities would seem to be a direct depressant action of the sex hormones on the musculature of the biliary tract. That these hormones do affect the smooth muscle of various organs is known in the case of the uterus and uterine tubes, and is strongly suggested in the case of the urinary bladder (4) and small intestine (5). The two important mechanisms in gall bladder evacuation *per se*, are the gall bladder muscle itself and the sphincter mechanism at the choledocho-duodenal junction. In the further investigation of this problem, it seemed desirable to determine, if possible, the effect of pregnancy and its hormones on both of these

[2] In addition there is some evidence to indicate that this effect disappears between the 3rd and 6th day of the puerperium.

mechanisms. The guinea pig was used throughout these experiments and our objects have been essentially two-fold: A, to test the *isolated* gall bladder of non-pregnant and pregnant animals and of animals injected with sex hormones; and B, to test the sphincter mechanism of normal and pregnant animals.

Response of the Isolated Gall Bladder of Pregnant, Non-pregnant and Sex-hormone-injected Guinea Pigs to a Standard Dose of Cholecystokinin. Method. Following decapitation of the animal, the gall bladder was removed, the cystic duct cannulated and the organ suspended in a constant temperature bath at 38° according to the method of Doubilet and Ivy (6) for measuring changes in intravesicular pressure. These authors noted that the optimal intra-gall bladder pressure for an optimal contraction in response to a submaximal dose of the hormone varied from 4.0 to 5.5 cm. of Sollman-Rademaekers' solution placed in the isolated vesicle of the guinea pig. In our experiments, therefore, the intravesical tension was regulated at this level before CCK was introduced. The same preparation of CCK was used which has been previously described; 0.25 mgm. of this preparation, added to the 50 cc. bath caused almost all gall bladders to contract submaximally and this dose was used throughout these experiments. At least 3 tests were made on each gall bladder. The response was recorded in centimeters of S-R solution.

Five different groups of animals were tested in this manner: I. Non-pregnants—25 animals, 20 females and 5 males. II. Pregnants—26 animals. III. Castrates—10 animals. IV. Estradiol injected—10 animals. V.[3] Progesterone injected—10 animals.

Experiments on castrate animals were performed 7 to 10 days following operation. The animals of group IV were injected with 7 daily doses of 0.7 mgm. of estradiol, while in group V the total dosage varied from 17.5 mgm. to 35 mgm. progesterone divided into 7 daily doses. Seven of the 10 animals in group V received a total of 35 mgm. The hormone injections were begun approximately 7 days after castration, and the animal was used for the experiment 24 hours after the last injection.

Results. In table 6 are shown the mean elevations of intravesical pressure in centimeters of S-R solution as determined by 3 trials on each gall bladder.

Analysis of the mean elevations of pressure reveals no striking difference between the various groups and only in the case of groups I and IV is the difference statistically significant; the critical ratio in the latter instance is 2.32.

On the possibility that a difference between the response of the gall bladders of pregnant and non-pregnant animals, if it existed, would be more manifest on the first introduction of cholecystokinin, all of the first trials

[3] Progesterone and Estradiol were supplied through the courtesy of the Schering Corporation and the Ciba Pharmaceutical Company respectively.

were analyzed. It was found that while the response was consistently less in the 1st than in the 2nd and 3rd trials, only a small difference existed between the pregnants and non-pregnants in this respect, the difference being relatively less than that shown in the averages of all 3 trials, as listed in table 6.

In the case of 11 non-pregnant and 12 pregnant animals in the above experiment, pressure readings were taken at 30 second intervals after the introduction of CCK in order to ascertain the rate as well as the height

TABLE 6

	NUMBER OF ANIMALS	MEAN RISE OF INTRAVESICAL PRESSURE (CM. S-R SOL.) + S.E. OF MEAN
I. Non-pregnants	25	2.41 ±0.215
II. Pregnants*	26	1.89 ±0.286
III. Castrates	10	1.77 ±0.328
IV. Estradiol injected	10	1.73 ±0.201
V. Progesterone injected	10	1.98 ±0.404

* All except 2 were between 31st and 65th day of gestation.

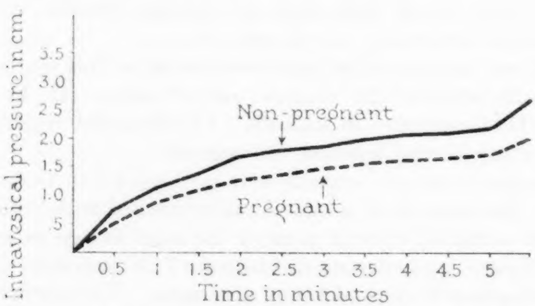

Fig. 1. These curves show graphically the average rate and height of the response of the gall bladder of non-pregnant and pregnant guinea pigs to a standard dose of CCK. The undesignated time shows the maximum response which occurred at some time after 5 minutes.

of response in these two groups. The averaged results are depicted graphically in figure 1.

Comment. A comparison of the response of the isolated gall bladder of pregnant and non-pregnant animals in table 6 fails to give conclusive evidence of a depressant action of pregnancy on the gall bladder muscle itself. The difference between these two groups (I and II in table 6) is not statistically significant (C.R. = 1.818), but the constant slight trend toward a decreased reactivity of the gall bladder muscle of the

pregnant animals makes it difficult to rule out this factor as a cause of decreased emptying power. Furthermore, an interpretation of the results must take into consideration the natural limitations of the *in vitro* experiment, one of which is the possibility of "washing out" of an inhibitory substance entirely or in part.

In the case of the castrate and hormone-injected guinea pigs, there is also a tendency toward diminished response of the isolated gall bladder. It is noteworthy that this tendency is greatest and the difference statistically significant, only in the case of the estradiol injected animals, in which the dosage of hormone was relatively large. This is an interesting result since estrone is generally conceded to increase the irritability of uterine smooth muscle.

It is our opinion that although pregnancy and the injection of progesterone and estradiol caused no marked decrease in reactivity of the *isolated* gall bladder, the observed trend indicates that such a decreased reactivity may be present at least to some degree *in vivo* and may be a factor in the decreased emptying power of the pregnant guinea pig gall bladder. This interpretation may be supported by the diminished evacuatory response of the gall bladder of the pregnant animal *in vivo* to cholecystokinin. This is not conclusive however because a diminished evacuatory response could also be due to increased sphincteric tone.

Intra-Sphincteric Pressure of Non-pregnant and Pregnant Animals. Method. Under sodium pentobarbital anesthesia, the abdomen was opened and the common bile duct ligated close to its union with the cystic duct. The common duct was then cannulated toward the sphincter of Oddi and the abdomen closed. After a recovery period of 1 hour, the cannula was slowly filled with S-R solution until the fluid level fell, which usually occurred suddenly, indicating escape of fluid into the duodenum. This fall usually continued for several minutes after which time the fluid column stabilized. Two readings were taken, the first, at the initial fall of the fluid column which represented the pressure at which the solution entered the duodenum, and the second reading, after the flow had stopped and the fluid column had stabilized itself, representing the mechanical pressure maintained by the sphincteric-duodenal mechanism, and which had to be overcome before further emptying into the duodenum occurred. Three tests were made in each animal, the first reading being taken only on the first test as it was only then that definite values could be obtained.

Results. The averaged results are shown in table 7.

Comment. There are only small differences between the two mean pressure readings in the pregnant and in the non-pregnant animals, the differences not being significant in either case. It is the pregnant animals which yielded the slightly greater values, and it is a matter of conjecture whether or not this represents an actual tendency toward a heightened

sphincteric pressure in the intact pregnant animal. In any event, as determined by this type of experiment, the mechanical pressure maintained by the sphincter mechanism at the choledocho-duodenal junction is not significantly altered in the pregnant animal from the non-pregnant, and seems to give *prima facie* evidence that increased sphincteric resistance is not a significant factor in the decreased emptying of the pregnant guinea pig gall bladder. These animals however were anesthetised.

Discussion. The influence of pregnancy *per se* on gall bladder volume is a question of some interest. Boyden (1) has stated that the pregnant gall bladder is larger than the non-pregnant, basing his conclusion on a study of 5 patients on whom cholecystograms were made during and after pregnancy. In our study of the guinea pig a rough correlation was found to exist in both pregnant and non-pregnant groups between body weight and gall bladder volume. A comparison of the animals of each group having similar body weights showed practically no difference in gall bladder size. Weights of pregnant animals included the products of conception in all cases. Whether the greater size of the pregnant gall bladder is due

TABLE 7

	NUMBER OF ANIMALS	PRESSURE (CM. OF S-R SOL)	
		1st reading (Mean)	2nd reading (Mean)
		cm.	*cm.*
Non-pregnant	12	9.83	5.14
Pregnant	12	10.25	5.99

primarily to a non-specific increase in body weight is difficult to state definitely, but our work with the guinea pig seems to indicate that such is the case. This question needs further investigation, preferably using the method of testing the same individuals before, during, and after pregnancy.

Impaired or delayed emptying of the pregnant gall bladder in the human (1), guinea pig and striped gopher (2), has been reported and quantitative evidence submitted in the case of the human (1). That delayed evacuation occurs also in the guinea pig is borne out by our results on the intravenous injection of cholecystokinin. In the dog the effect of pregnancy on gall bladder evacuation is uncertain in our opinion. In the cat according to Whitaker and Emerson (8), pregnancy itself exerts little or no influence on gall bladder emptying. In the latter animal however, Dubois and Hunt (9) have shown that even among non-pregnant members of the species, there is considerable individual difference in the response of the gall bladder to a meal of egg yolk and cream.

Although there are anatomical and physiological differences in the biliary

system of different species, there are, theoretically, three general ways in which uncomplicated pregnancy could bring about an alteration of gall bladder motility. These may be listed, as follows, along with the possible underlying mechanisms: I. *Interference with* (a) *Production*, or (b) *Transport of cholecystokinin*, e.g., *1*, by an alteration of gastric emptying, or *2*, by chemical inactivation of the hormone in transport. II. *Inhibition of gall bladder muscle.* III. *Increased resistance of sphincteric-duodenal mechanism.*

Effects II and III may be produced by: (a) *mechanical factors:* enlarged uterus, and altered intra-abdominal pressure; (b) *changes in amount, physical or chemical character of bile;* (c) *nerve induced disturbances:* Autonomic nervous system and reflexes originating from uterus and pelvic organs, and the G-I tract, especially the colon; (d) *Humoral substances:* Sex hormones and non-specific chemical substances.

Although the effect of pregnancy on fundamental physiological processes has, in a few instances, for example, those dealing with sex hormones and composition of bile, been studied at some length, very little is known of any direct effect of these altered processes on gall bladder activity. That evidence which is available has been summarized in two recent articles (1, 7) and will not be recounted here.

Our experiments with the isolated gall bladder of pregnant and sex-hormone-injected animals demonstrate only a trend toward an actual decreased reactivity of the gall bladder muscle itself. Likewise the measurement of sphincteric resistance indicates only a slightly increased resistance of the sphincteric-duodenal mechanism in pregnant animals. While it is probable that these factors, singly or together, may be at least partially responsible for impaired emptying of the gall bladder in the intact guinea pig, we believe that these results suggest the existence of another factor, namely a partial inactivation of cholecystokinin in transport. It is to be emphasized, however, that no direct evidence is available at present to substantiate or repudiate this view. It emerges as a possibility for investigation due to a lack of any marked inhibition of the isolated gall bladder or of marked increase of sphincteric resistance in the pregnant guinea pig, and it would seem to be compatible with the temporary refractoriness of the fetal gall bladder to CCK immediately following delivery.

SUMMARY

1. Previous studies have indicated that in the human, guinea pig, striped gopher and dog, the gall bladder of pregnant animals does not empty as well as that of non-pregnant animals in response to a meal of egg yolk and cream.

2. Using the guinea pig as the experimental animal, *in vitro* and *in vivo* experiments were carried out in order: *a*, to test the statement that the

pregnant guinea pig gall bladder does not empty as well as the non-pregnant, and *b*, to study the mechanisms involved.

3. In the guinea pig, feeding experiments seem unreliable as a means of detecting differences in degree of emptying of the pregnant and non-pregnant gall bladder, though the results tend to indicate that the viscus of the pregnant animal emptied more slowly.

4. Intravenous injection of 0.5 mgm. cholecystokinin produced prompt and efficient emptying of the non-pregnant guinea-pig gall bladder, as tested in 18 animals, but a delayed and much less effective emptying of the gall bladder of 16 pregnant animals. Some evidence was obtained which indicates that between the 3rd and 6th day of the puerperium, the gall bladder response to CCK returns to normal.

5. In guinea pigs of similar body weights, there was little or no difference in the mean gall bladder volumes of 15 pregnant and 15 non-pregnant animals, when the weight of the products of conception is included.

6. In a series of *in vitro* experiments, the response of the isolated gall bladder of 25 non-pregnant, 26 pregnant, 10 castrate, 10 estradiol-injected, and 10 progesterone-injected animals was tested to a standard dose of CCK. The response was measured in centimeters of intra-gall bladder pressure. In the pregnant, castrate and both hormone-injected groups, the mean values indicated a somewhat diminished response of the gall bladder compared to the non-pregnant control groups. This diminution of response was most marked and statistically significant only in the case of the estradiol-injected animals.

7. The mean resistance of the choledocho-duodenal mechanism was found to be only slightly greater in 12 pregnant animals than in 12 non-pregnant. The difference was not statistically significant.

8. The small differences in the direction of decreased reactivity of the gall bladder muscle and increased resistance of the sphincteric mechanism are, apparently, in part responsible for the impaired emptying of the gall bladder of the pregnant guinea pig.

9. The gall bladder of the guinea pig fetus near term is refractory to cholecystokinin injected into the mother or into the fetal circulation. Three to four hours after delivery the gall bladder responds.

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THE RÔLE OF THE ADRENAL CORTEX AND THE ANTERIOR PITUITARY IN DIABETES INSIPIDUS[1]

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Since the clinical observations of von Hann (1) and the experiments of Richter (2), it has been well confirmed that the presence of a functional anterior lobe as well as the inactivation of the posterior lobe of the pituitary is essential for the maintenance of a permanent, maximal diabetes insipidus. The nature of this anterior-lobe activity has yet to be precisely defined. A specific anterior-lobe diuretic factor has not been isolated, and it is probable that the action is an indirect one. Thyroid maintenance may be involved to some extent through its action on metabolism. The maintenance of the appetite in general by the anterior lobe and the level of the salt intake in particular have been considered as contributing factors. The literature concerning this problem has been well considered in the reviews of Fisher, Ingram and Ranson (3) and Gersh (4).

Silvette and Britton (5, 6), on the basis of indirect evidence, concluded that the factor responsible for the influence of the anterior lobe on diabetes insipidus was support of adrenal cortical function. At the time their work appeared we were investigating this same possibility; and also studying the question of the supposed refractoriness of the hypophysectomized rat to the expected diuretic effects of anterior-lobe replacement therapy. The results of these and related experiments are presented here. An abstract of the earlier experiments has been published (7).

Methods. In the chronic experiments male rats weighing 150 to 200 grams were used. Continuous daily records were made of body weight, food intake, water intake and urine output through observation periods which were usually of several weeks' duration. In most cases such determinations were also made a week before hypophysectomy for control purposes. Experimental conditions were similar to those previously described (8). Ten per cent sucrose, 1 per cent dry yeast and 5 grams fresh lettuce daily were added to our standard diet (9). Acute tests for antidiuretic sub-

[1] This work was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

stances were made by the Burn technique as modified by Heller and Urban (10). Crude anterior-lobe extracts (APE) were made by grinding fresh chilled or frozen glands in sand and centrifuging the debris from a saline suspension. This material, kept solidly frozen at $-5°C.$, was found to be active for several weeks.

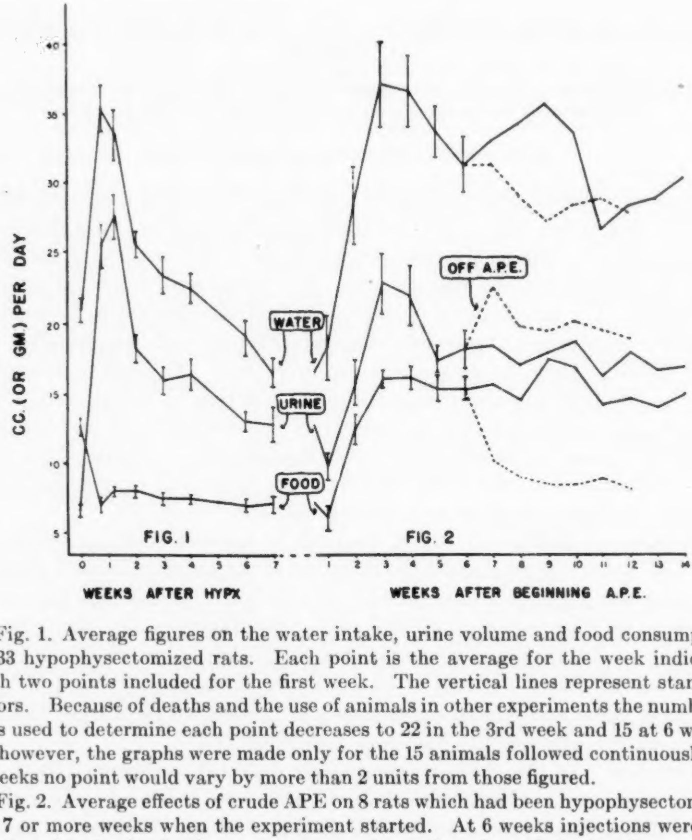

Fig. 1. Average figures on the water intake, urine volume and food consumption of 33 hypophysectomized rats. Each point is the average for the week indicated with two points included for the first week. The vertical lines represent standard errors. Because of deaths and the use of animals in other experiments the number of rats used to determine each point decreases to 22 in the 3rd week and 15 at 6 weeks. If, however, the graphs were made only for the 15 animals followed continuously for 6 weeks no point would vary by more than 2 units from those figured.

Fig. 2. Average effects of crude APE on 8 rats which had been hypophysectomized for 7 or more weeks when the experiment started. At 6 weeks injections were discontinued in 4 animals whose average records are then shown in broken lines.

The completeness of anterior lobe removal was checked by gross observations at autopsy, by adrenal and gonad atrophy, by a chronic loss of weight, etc.

Effects of hypophysectomy. In general the nature of the alterations in water exchange following hypophysectomy in the rat reported by others have been confirmed. Some facts summarized in figures 1 and 3 necessi-

tate further comment. Because of the inevitably high error of urine volume measurements on small animals, results are frequently reported on the basis of water intake alone. This practice may give misleading results. On the basis of water intake, our figures would confirm the frequent statement that diabetes insipidus after total hypophysectomy in the rat is only transitory, since thirst eventually returned to normal levels or below.

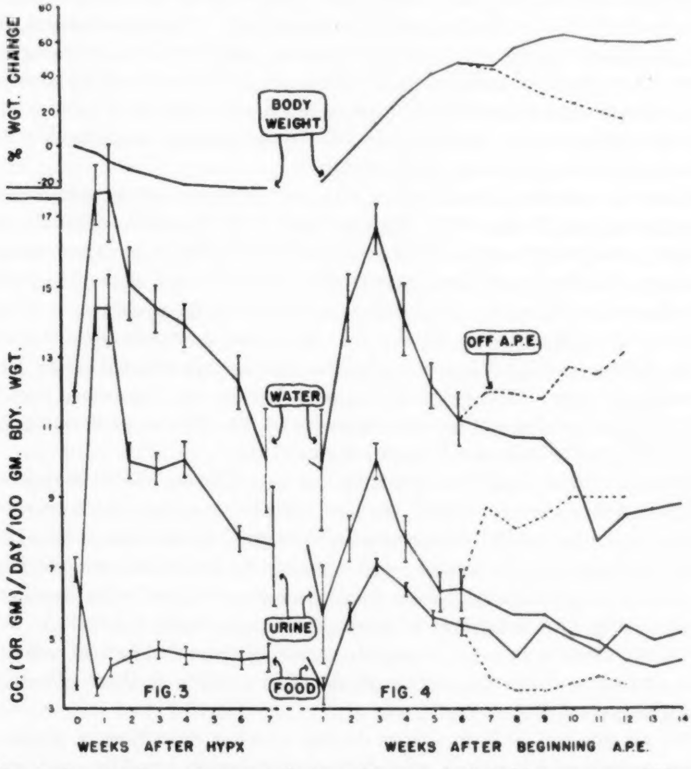

Fig. 3. Same as figure 1, except that results are calculated in grams per 100 grams' body weight. A curve of body weight change is also included.

Fig. 4. Same as figure 2, calculated as explained for figure 3.

With but rare exceptions, however, urine volumes remained at approximately twice normal levels; and a return to normal was generally associated with moribundity, even in animals on which observations were made for much longer periods than shown in figures 1 and 3. The diabetes insipidus induced by hypophysectomy in the rat is therefore quickly reduced in intensity but in an attenuated state the polyuria at least is apparently permanent. Such results are also apparent in the figures of

Dodds et al. (11). The fact that the food intake of these animals was about half normal, and the metabolism no doubt greatly reduced—although it was not measured—enhances the significance of this continued polyuria. Such results also suggest that the hypophysectomy technique used here, for functional purposes at least, effectively eliminated the neurohypophysis.

Phillips and Gilder (12) have also emphasized the permanence of diabetes insipidus in the hypophysectomized rat. They considered, however, that this finding is frequently obscured unless the water exchange is expressed in terms of body weight. This was not true in our hypophysectomized untreated animals; but in treated animals, described below, where excessive growth was induced, absolute figures differ considerably from those expressed in terms of body weight.

Others have found that a high initial polydipsia is not invariable after hypophysectomy in the rat. Richter thinks (2) that this depends upon variable remaining remnants of the neurohypophysis or upon surgical mishaps. Marked polydipsia occurred in about 80 per cent of our cases, but even in its absence a mild polyuria was generally present.

Effects of crude pituitary extract. It has been shown in several species that a near-maximal diabetes insipidus can be maintained after hypophysectomy when APE is given (3, 4). In the rat, however, negative results have attended similar experiments (13, 14, 15) except in one animal (case 107J) of Richter and Echert's series (15).

Eight rats which had been hypophysectomized from 46 to 100 days and in which water exchange had become stabilized at typical levels were injected with crude APE—equivalent of 0.16 gram fresh anterior-lobe tissue daily—for from 6 to 14 weeks. (In addition to the effects reported here the extract would also induce in small dosage a marked enhancement of the glycosuria and ketonuria of partially depancreatized ferrets.) With these doses there was not a complete repair of the atrophic reproductive tracts and adrenal cortices although detailed studies of these effects are lacking as yet.

After an interval of 3 or 4 days during which a reduction of water exchange occurred, a marked stimulation of growth, appetite and water exchange followed in all cases. Water intake reached maximal diabetes-insipidus levels within 3 weeks and then declined. The decline was gradual when expressed in absolute terms, but because of the rapid weight gains was abrupt when expressed in terms of body weight. Urine volumes increased but not to maximal diabetes-insipidus levels. This disproportionate increase in water intake was probably due to a water retention associated with rapid growth. Burn tests indicated some antidiuretic substance in the extract but not in amounts sufficient, had it been pituitrin, to affect daily water exchange in the dosage used here.

By the 5th to 6th week of treatment the secondary decline of water exchange was such that, expressed as a percentage of body weight, it was back to near pre-treatment levels and later dropped still further. The decline was less marked when expressed in absolute terms. This drop in fluid exchange may have been due to an anti-hormone type of refractoriness, but if so it developed only for those factors which maintained water exchange, and not for those which concerned growth and appetite, both of which remained at abnormally high levels.

After six weeks, injections were discontinued in half of the animals and continued in the others. Cessation of treatment evoked a rapid loss of weight and appetite, an increase in urine volume and a slight drop in water intake which was probably insignificant. The augmentation of urine volume was perhaps due to the rapid loss of tissue water coincident with weight depletion. The maintenance of water intake (or its rise in terms of body weight) occurred simultaneously with a sharp reduction in food consumption. This also indicates that the food intake (or the salt content thereof) is not, as some have suggested, a factor of decisive importance in determining the water exchange. These experiments, however, were not designed to furnish critical evidence on this point.

Effects of adrenotropic hormone.[2] Preliminary experiments on 10 animals indicated that the decline in the high diabetes insipidus which follows hypophysectomy could not be prevented by giving adrenotropic hormone (in combination with lactogenic hormone) immediately after operation, in amounts which maintained adrenal weights at above-normal levels (7).

We next used animals that had been hypophysectomized for some time, and in which the initial high water exchange had subsided to see if a diabetes insipidus could be restored. The periods immediately before and after treatment in each case furnished control figures, and were of 4 or more days' duration. A purified adrenotropic preparation was given in doses of either 4, 5 or 10 mgm. per day—amounts adequate to repair adrenal weights to normal after about 7 days' treatment. Since the batches of adrenotropin had different potencies, the effective dosages were approximately identical.

In every instance the effect was clearly to decrease rather than increase the water exchange. The typical ratio of urine volume to water intake seen in untreated hypophysectomized rats remained throughout.

The nature of the antidiuretic action of the adrenotropin is not clear. We found that it was also antidiuretic in the Burn test in intact animals and considered, therefore, that it probably was contaminated with pituitrin. Assays for pressor and oxytocic activity carried out in the research laboratories of Parke, Davis & Co., however, indicated that only negligible

[2] The authors are indebted to Dr. Oliver Kamm, Parke, Davis & Co., for the andrenotropic and posterior-lobe preparations used here, and also for the assays of the pressor and oxytocic content of the adrenotropic material.

amounts of these substances were present—less than 0.1 pressor and 0.025 oxytocic unit in our daily adrenotropin doses. Injections of pitressin and pitocin in these daily amounts gave slight, if any, effect on the daily water exchange of hypophysectomized animals (table 1). Two units of pitressin per day, an amount 20 or more times that in the adrenotropin, were necessary to depress water exchange as the adrenotropin had done (table 1). Furthermore, the method of preparing the adrenotropic extract was such as to destroy largely its pressor and oxytocic activity. It is possible, if the existence of a questionable separate posterior-lobe antidiuretic hormone is granted, that such a contaminant may have been the active substance.

According to the principles described by Kárady et al. (16) the chronic antidiuretic activity of this material would not have been due to a nonspecific stimulus to antidiuresis seen in the "alarm reaction." Furthermore, the mere presence of a foreign substance does not account for the antidiuresis in the Burn technique as our APE was antidiuretic by this test but a similar muscle extract was not.

Whatever may have been the source of this antidiuresis after adrenotropin injections, the experiments described below make it doubtful that the action was mediated through the adrenal cortex. Neufeld et al. (17) have also recently observed an antidiuretic anterior lobe product.

Effect of adrenal cortical extract. In experiments similar to the above, cortical extract was injected into hypophysectomized rats to observe its influence on the water exchange. Salt-free extracts, made in the laboratory of Dr. W. W. Swingle, were used. Injections were in divided doses—3 cc. per day. This dose was about 6 times that needed for normal growth and appetite in adrenalectomized rats of similar size and age. It was a dose adequate for full protection of adrenalectomized animals during the acute stress of intoxicating doses of water. Larger doses were avoided because of the possible complications of adrenal cortical overdosage phenomena. As seen from table 1, there was no significant effect on the water exchange. The variations were within normal range.

Similarly in two cases desoxycorticosterone acetate had no effect in doses of 1 mgm. per day (table 1). The decline in water exchange observed in these cases was not unusual for the three weeks for which data are tabulated.

We believe that the doses of cortical extract used here are more than adequate for replacement of cortical function in rats under optimal living conditions. If so, it is clear that the action of the anterior lobe in maintaining a maximal diabetes insipidus is not effected through the adrenal cortex.

Discussion. In all mammals which have been investigated some influence of the anterior lobe is essential for the maintenance of a high

TABLE 1

Effects of various substances on the water exchange of hypophysectomized rats

CASE NUMBER	NUMBER OF DAYS INJECTED	BEFORE TREATMENT		DURING TREATMENT		AFTER TREATMENT	
		Water	Urine	Water	Urine	Water	Urine
			Adrenotropic hormone				
1	9	29	22	14	11		
2	6	33	26	20	15	26	24
3	7	35	32	16	13	23	22
4	9	29	22	19	15		
5	4	16	10	10	7	16	13
6	8	26	17	15	10	18	13
7	4	23	17	14	10	25	20
Average............		27.3	20.9	15.4	11.6	21.6	18.4
			Pitressin (0.1 U/day) and pitocin (0.025 U/day)				
8	3	17	13	21	12	22	13
9	3	22	13	19	10	18	13
10	6	18	13	14	11	17	13
11	3	21	14	10	7	13	6
12	6	20	12	15	8	15	8
13	3	28	21	31	22	19	13
14	3	19	13	14	10	18	10
15	6	18	10	11	9	17	11
16	6	20	16	14	12	17	13
Average............		20.3	13.9	16.6	11.1	17.3	11.1
			Pitressin (2 U/day)				
17	3	31	22	21	16	27	23
18	3	31	22	16	12	29	22
19	3	33	29	22	15	31	21
Average............		31.7	24.3	19.7	14.3	29.0	23.0
			Cortical extract (3 cc./day)				
20	7	27	24	22	21	26	23
21	6	18	19	17	19	21	20
22	7	32	27	23	21	21	17
23	5	24	17	14	12	16	10
24	5	30	20	25	20	26	17
25	5	22	16	21	20	25	20
26	5	27	20	32	30	32	26
Average............		25.7	20.4	22.0	20.4	23.9	19.0
			Desoxycorticosterone acetate (1 mgm./day)				
27	7	21	15	20	16	20	16
28	7	20	13	17	12	15	11
Average............		20.5	14.0	18.5	14.0	17.5	13.5

diabetes insipidus after ablation or inactivation of the posterior lobe. The results presented here show that the rat is no exception to the rule that anterior lobe replacement effects a heightened water exchange after hypophysectomy. This action of APE in rats is, at least in its maximal aspect, only transitory. The effects of the adrenal cortex on electrolyte and water metabolism invite the hypothesis that the anterior lobe's action in supporting diabetes insipidus is in part its well-known one of maintaining cortical function. Such a possibility was favorably considered on theoretical grounds in the monograph of Fisher, Ingram and Ranson (3), and definitely stated as a theory by Silvette and Britton (5, 6). The data presented here negate this theory. The action of adrenotropic hormone was, if anything, to depress water exchange of hypophysectomized rats; adrenal cortical preparations had no effect; but crude APE reëstablished a maximal diabetes insipidus. Furthermore, other work has shown that under certain conditions the cortical hormone is antidiuretic rather than diuretic (8). Other aspects of the possible interrelationship between the posterior pituitary and the adrenal cortex have been studied by Winter, Ingram and Gross (18).

SUMMARY

1. A polyuria, but not necessarily a polydipsia, is maintained for seven weeks or longer in hypophysectomized rats and is terminated only by moribundity. Water intake alone is not an accurate measure of the altered water exchange of hypophysectomized rats.

2. Contrary to other reports, anterior-pituitary extract will consistently restore diabetes insipidus in long-term hypophysectomized rats to its maximal post-operative levels. Such results are in harmony with those from other species. This effect was not clearly related to growth and appetite responses.

3. The influence of the anterior lobe in maintaining a maximal diabetes insipidus is not mediated through the adrenal cortex. Adrenotropic preparations were antidiuretic in long-term hypophysectomized rats; they did not prevent the subsidence of the high initial diabetes insipidus which follows hypophysectomy; and salt-free adrenal cortical extract or desoxycorticosterone acetate had no effect.

4. The antidiuretic action of adrenotropic preparations, exhibited also in the Burn assay in intact animals, could not be accounted for by their titer of posterior-lobe pressor or oxytocic substances.

ADDENDUM

While this paper was in press two significant articles appeared reporting the production of a polydipsia-polyuria syndrome, accompanied by disturbances in electrolyte metabolism, in normal and hypophysectomized rats (19) and intact dogs (20) receiving large doses of desoxycorticosterone. Using comparable doses we have subsequently obtained similar results on water exchange of hypophysectomized

rats. Such results are not clearly applicable to the problem under discussion here because the doses used (4–8 mg. per day in rats) were almost certainly outside the range of the cortical hormones secreted by the animal with diabetes insipidus even with the anterior lobe intact (see text, p. 146). Ragan *et al.*, in fact, distinguished their syndrome from that of diabetes insipidus.

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THE PASSAGE OF THIOCYANATE AND GLUCOSE FROM THE BLOOD STREAM INTO THE JOINT SPACES[1,2]

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Previously reported investigations of synovial membrane permeability have concerned mainly the passage of various substances from the joint spaces into the blood stream or lymphatics (1) (2) (3), although passage in the opposite direction is equally important. Quantitative information relative to the transfer of proteins from the vascular system to normal joints and other body cavities has been reported by Bennett and Shaffer (4). The present experiments were undertaken to obtain similar data concerning the passage of two crystalloid substances, CNS ions and glucose.

METHODS. Normal calves were used for all experiments. The animals were bound in a manner which kept them as nearly motionless as possible. That they were not disturbed by the restraining measures was shown by the fact that they frequently slept during the experiments. The synovial fluid aspirations were done aseptically following novocain infiltration of the overlying skin. All synovial fluid specimens were collected from joints proximal to the bindings.

Either 20 to 50 cc. of sterile 5 per cent NaCNS solution or 100 to 155 cc. of 25 per cent glucose solution was injected as rapidly as possible into one external jugular vein. The blood samples were obtained from the opposite jugular vein. Samples of blood and synovial fluid were withdrawn over periods varying from 1 to 26 hours. When periods over 2 hours intervened between the collection of samples, the animals were allowed to walk during the intervals. In such instances water drinking was permitted.

The amount of synovial fluid which could be aspirated usually varied from 0.5 to 3 cc. At times the tarsal joints yielded as much as 6 cc. Although a few of the calves may have become dehydrated during the journey to the abattoir, it was always possible to obtain sufficient fluid for analyses. The amount aspirated at any one time was usually 1 cc., thus leaving some fluid for subsequent removal.

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[2] The expenses of this investigation were defrayed, in large part, by a grant from the Commonwealth Fund.

[3] Nemours Foundation Fellow, 1938–1939.

[4] Rockefeller Fellow (Medical Research Council of Great Britain), 1937–1938.

Most of the specimens were free from blood. Those which were grossly bloody were discarded; slightly blood-tinged samples were analyzed.

Thiocyanate determinations were made by a slight modification of the method of Lavietes, Bourdillon and Klinghoffer (5), which permitted dilution of the serum and synovial fluid specimens, since at times the latter were less than 1 cc. in volume. We found, as did Lavietes and his co-workers, that duplicate determinations agreed within 3± per cent.

The following chemical methods were used: nonprotein nitrogen, Folin and Wu (6); chloride, Eisenman modification of the Van Slyke method (7); sugar, Folin (8). The protein was determined by a modified macro-Kjeldahl method. The difference between the total nitrogen and nonprotein nitrogen was multiplied by the factor 6.25 to obtain the value for total proteins.

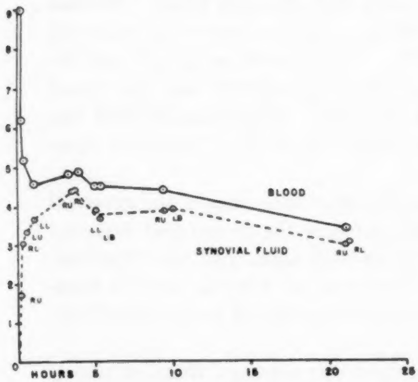

Fig. 1

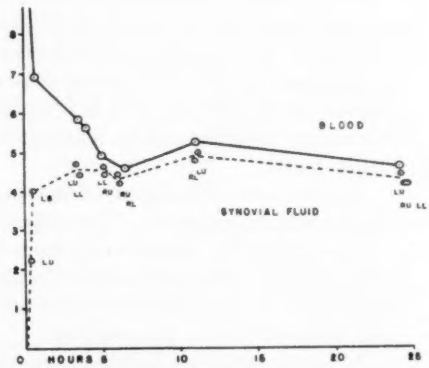

Fig. 2

Fig. 1. Calf weighed 173 lbs. Serum and synovial fluid CNS levels following the injection of 28 cc. of 5 per cent NaCNS at 0 hour. Ordinates, milligram NaCNS per 100 cc. *LU* and *LL* indicate upper and lower portions of left carpal joints. *LB* indicates left tarsal joint. *RU*, *RL* and *RB* indicate similar joints on the right.

Fig. 2. Calf weighed 134 lbs. Serum and synovial fluid CNS levels following the injection of 20 cc. of 5 per cent NaCNS at 0 hour. Ordinates, milligram NaCNS per 100 cc.

Results. *Thiocyanate experiments.* In 7 experiments the blood and synovial fluid CNS levels were determined at intervals up to 26 hours; in 5 others synovial fluid was removed, as nearly simultaneously as was possible, from a number of joints, in order to determine whether the rate of entry into different joints was the same.

Figures 1 and 2 show that during the first hour following the intravenous injection of NaCNS, there occurred a very rapid rise in the synovial fluid CNS concentration and a correspondingly rapid fall in the blood concentration. The blood curves then flattened and demonstrated a fairly constant slope for the duration of the experiments. The synovial fluid CNS rose more slowly and finally assumed a constant relationship to the

blood CNS. The time required for attaining such equilibrium varied considerably. In the experiments illustrated (figs. 1 and 2), it was between 1 and 4 hours and 4 and 5 hours, respectively. In another experiment equilibrium was present in 60 to 70 minutes, and in 4 others it was demonstrable within 2 hours. The longest time required for the attainment of equilibrium was over 6 hours.

The slight increase in the CNS concentration of both the serum and synovial fluid which occurred between the fifth and eleventh hours, as shown in figure 2, is difficult to explain. That it was not due to experimental errors is shown by the unaltered relationship of the serum and synovial fluid CNS levels at the fifth, sixth and eleventh hours. Similar unexplained rises in the blood CNS level have been observed in man and the horse by Crandall and Anderson (9). These workers found that the lymph and gastric secretion of dogs contained more CNS than the blood serum for periods up to 4 hours after injection. It is possible that the secondary rises are due to subsequent rediffusion of the CNS from these body fluids.

The serum CNS concentration remained higher than that of the synovial fluid in all experiments. The difference between the serum and synovial fluid equilibrium levels varied from 0.25 to 0.50 mgm. per cent NaCNS. The average difference was 0.37 mgm. per cent, or 8.8 per cent, in 8 experiments in which the blood was not collected under oil and in which apparent equilibrium was reached.

In one experiment sufficient synovial fluid was obtained to allow simultaneous determinations of CNS, protein and chloride (see table 1). Since the blood specimens were taken under oil, the CNS values expressed in terms of milligrams per 100 cc. of water approximate more exactly those existing *in vivo* than in the other experiments.

The relative concentration of CNS in the cistern fluid, aqueous humor and joints is shown graphically in figure 3. It was lowest in the cistern fluid. This observation is in agreement with that of Crandall and Anderson (9), who found only traces of CNS in the spinal fluid of dogs, 24 hours after intravenous injection. Higher concentrations of CNS were found in the aqueous humor of both eyes. The content of the synovial fluid specimens was even higher and of the magnitude found in previous experiments of like duration. The difference in the serum CNS concentration of the samples obtained in the twentieth and twenty-first hours is far greater than would ordinarily be expected. That the fall in concentration was due to an increased extracellular fluid volume is suggested by the finding of lowered protein and chloride values in the second serum sample (see table 1).

Differences in the rate of entry of CNS into various joints of the same animal could not be consistently demonstrated. In a few instances the

tarsal joints contained less CNS than other joints aspirated simultaneously. The results suggest that equilibrium may be attained more slowly in the tarsal joints, but the number of determinations before the attainment of equilibrium is insufficient to prove this.

In some of the experiments of longer duration, the same joints were of necessity aspirated two or three times. Occasionally, the second or third synovial fluid specimen was cloudy. Smears from such fluids showed numerous polymorphonuclear leukocytes, indicating that the previous aspirations had caused irritation of the synovial tissues. In most cases, these abnormalities were without effect on the CNS levels.

TABLE 1

SPECIMEN	TIME AFTER INJECTION	TOTAL PROTEIN	H_2O	CNS		CHLORIDE		DONNAN RATIO CNS	DONNAN RATIO Cl
				Mgm. per 100 cc.	Mgm. per 100 cc. H_2O	M.eq. per 1000 cc.	M.eq. per 1000 cc. H_2O		
		grams per 100 grams	*grams per 100 cc.*						
Serum 1	19°41′	5.62	94.82	6.50	6.86	99.35	104.7		
Serum 2	20°45′	5.30	95.09	5.72	6.02	94.96	99.8		
Joint 1, L.U.	19°46′	1.39	98.42	5.46	5.54	102.9	104.6	1.225	0.998
Joint 2, L.L.	19°49′	1.37	98.44	5.87	5.96	104.0	105.6	1.132	0.986
Joint 3, L.B.	19°51′	1.08	98.68	5.75	5.82	106.7	108.1	1.156	0.962
Joint 4, R.U.	20°04′	1.38	98.43	5.63	5.72	107.3	108.9	1.146	0.946
Joint 5, R.L.	20°06′	1.43	98.38	5.19	5.28	103.5	105.2	1.236	0.978
Joint 6, R.B.	20°10′	1.12	98.65	5.84	5.92	104.8	106.3	1.094	0.965
Cistern fluid	20°30′	0.01	99.59	<.05	<.05	121.0	121.0	121.7	0.828
Right eye	21°15′			1.84					
Left eye	21°15′			1.77					

Data obtained in one experiment after the intravenous injection of 50 cc. of 5 per cent NaCNS 42 hours before, and 26 cc. of 5 per cent NaCNS 19 hours and 41 minutes before. The blood CNS and Cl values used in the Donnan ratios were interpolated from the initial and final blood values as determined, assuming that the fall of Cl and CNS was constant.

The available fluid (extracellular) values for 4 calves calculated by the method of Crandall and Anderson (9) varied from 15.7 to 24.4 per cent of the body weight, which is lower than the figure obtained in humans (5), and may indicate that some of the animals were dehydrated when the experiments were performed.

Glucose experiments. Two glucose experiments were performed. In figure 4, the data from the second experiment are presented graphically. The synovial fluid glucose rose more gradually than the synovial fluid CNS. In the first experiment the initial rise of synovial fluid sugar lagged behind that of the blood by at least 20 minutes, differing in this respect

from CNS which could be detected as early as 9 minutes after injection. The blood sugar in both experiments returned almost to the pre-injection levels in less than 2 hours. When the serum glucose fell, the synovial fluid glucose again showed a lag. Similar results in patients with effusions of the knee joints have been observed (10) (11).

Discussion. A constant serum concentration of CNS has been considered by most workers to indicate the attainment of diffusion equilibrium (5) (9) (12). In normal men and dogs and in some of these experiments,

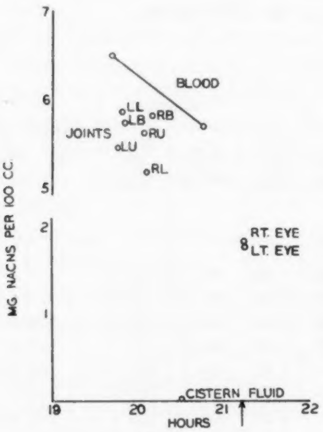

Fig. 3

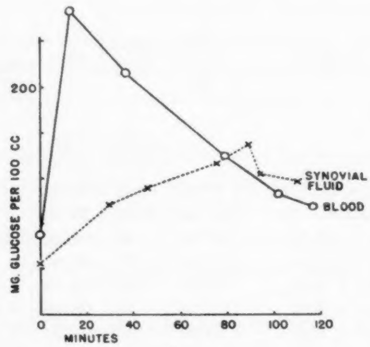

Fig. 4

Fig. 3. Calf weighed 208 lbs. Thiocyanate concentration in blood serum, synovial fluid, aqueous humor and cistern fluid following the intravenous injection of 50 cc. of 5 per cent NaCNS 42 hours before, and 26 cc. of 5 per cent NaCNS 19 hours before. Abscissae expressed in hours after the second injection. The arrow indicates the time when the animal was sacrificed by section of the large vessels in the neck. Eye fluid was collected immediately afterwards.

Fig. 4. Second glucose experiment. Calf weighed 169 lbs. Injection of 155 cc. of 25 per cent glucose solution at 0 minute. The glucose concentration in serum and synovial fluid for the following two hour period is shown.

equilibrium, as determined this way, is usually reached within one-half to one hour. Despite the initial rapid rate of entry of CNS into the joint spaces of calves, the time at which diffusion equilibrium between serum and synovial fluid is attained is considerably longer than one-half hour and is also quite variable. For example, in one experiment (fig. 1) a relatively constant serum concentration was reached between 43 and 72 minutes, but true equilibrium between serum and synovial fluid was not demonstrable at this time.

The greater volume of fluid (synovia) in the tissue spaces (the joints)

is responsible here for a situation somewhat similar to that found in human patients with edema, in whom equilibrium between plasma and transudates in the subcutaneous tissue, the thoracic and the abdominal cavities usually is not attained until 6 to 10 hours after the intravenous administration of NaCNS (13). Gilligan and Altschule interpret their data from patients as follows: "The time required for attainment of diffusion equilibrium between plasma and edema fluid in a given compartment appears to be directly proportional to the volume of transudate in that compartment" (13).

Their data indicate that at diffusion equilibrium the concentration of CNS in transudates varies directly with the protein concentration and like those of Lavietes, Bourdillon and Klinghoffer (5) show that the average serum CNS concentration is always higher than that of transudates. These differences were thought to indicate that some of the CNS was "bound" to protein or some other relatively non-diffusible substance contained in plasma. Differences between the serum and synovial fluid CNS concentrations of approximately the same degree were observed in the present study.

The Donnan ratios for chloride and CNS are listed in table 1. The ratios for chloride are constant and are similar to those previously found for cattle synovia and other body fluids with the composition of dialysates (14). The CNS ratios vary considerably and average 19.2 per cent higher than those for chloride.

Differences in CNS content of the synovial fluid, the cistern fluid and the aqueous humor can probably be related to differences in protein content and the anatomical and physiological barriers intervening between the blood capillaries and these three body fluids.

Following the intravenous injection of glucose, factors other than simple diffusion of this substance through the body coöperate in rapidly lowering the blood sugar. When this occurs, the rate of utilization of glucose by the articular tissues and the rate of diffusion from the joint space into the synovial capillaries are not sufficiently rapid to keep the synovial fluid sugar level at or below that of the serum. Similar glucose differences between blood and pleural transudates in diabetic patients with cardiac failure have been described (15). Glucose equilibrium was not attained in the present experiments. The fact that glucose enters the joint space more slowly than CNS suggests that glucose equilibrium is reached later than is that of CNS. The validity of this assumption can only be tested by simultaneous experiments of longer duration on the same animal.

It is probable that substances resembling CNS and glucose in molecular size and physical composition, whether necessary for or detrimental to the economy of the intra-articular structures, can enter the joint spaces. Since previous experiments have shown that compounds of small molecular

dimensions are promptly transferred from resting joints to the blood stream (16), it is readily understood why normal synovial fluid is kept in balanced equilibrium with the blood. Further studies of this nature are necessary if a better understanding of articular physiology in health and disease is to be obtained.

CONCLUSIONS

1. Thiocyanate ions and glucose diffuse readily into the joint spaces of calves following intravenous injection, CNS entering more rapidly than glucose.

2. Thiocyanate diffusion equilibrium between serum and synovial fluid is usually attained in from 1 to 4 hours following the intravenous injection of NaCNS.

3. At equilibrium the concentrations of NaCNS in sera not collected under oil averaged 8.8 per cent higher than the synovial fluid concentrations.

4. Thiocyanate diffuses into the joint spaces in larger amounts than into the anterior chambers of the eyes. Only traces enter the cistern fluid.

5. These studies indicate that some of the CNS is held in the blood in a non-diffusible state.

6. With respect to CNS and glucose the equilibrium between serum and synovial fluid resembles the equilibrium between serum and transudates in patients with edema.

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THE RELATION OF THE Q-T INTERVAL TO THE REFRACTORY PERIOD, THE DIASTOLIC INTERVAL, THE DURATION OF CONTRACTION AND THE RATE OF BEATING IN HEART MUSCLE

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The present work was undertaken as a preliminary to the study of the action of drugs on the heart using the action potential (Q-T interval) as an index. It was considered desirable for this purpose to know the relation between the absolutely refractory period and electrical changes, the relation between electrical changes and the duration of contraction of the muscle, and the variation of electrical effects due to heart rate. We have studied these relations in strips of turtle heart and have made a few observations of the recovery of the Q-T interval in man after exercise. For convenience we have used the term Q-T interval for the interval between depolarization and repolarization of the heart strips in analogy to the common electrocardiographic nomenclature.

Various measurements indicate that the absolutely refractory periods of tissues are related to the intervals between the depolarization of the cell membranes consequent to excitation and the subsequent repolarization associated with recovery. Tait (1910) first seems to have suggested a connection between the refractory phase and the electrical manifestations. While this is agreed upon in general there is considerable disagreement as to the exact relation. Adrian, for example, (1921) concluded that the absolutely refractory phase corresponded closely with the duration of the monophasic action potential in frogs' nerve and heart but not in skeletal muscle. Gasser and Grundfest (1936) recently confirm Adrian's view for nerve. In the heart, however, it was indicated by earlier work of Trendelenburg (1912) and deBoer (1915) that the absolutely refractory period might outlast considerably the monophasic electric response. There are certain difficulties arising in all attempts at correlation. In the case of nerve in particular, it is not safe to assume that the monophasic action potential wave corresponds exactly to the period intervening between depolarization and repolarization at a certain region. In the case of the heart, additional uncertainties are introduced because not only does the

slowness of conduction enable different regions to be excited and to recover at quite different times, but it is well known that all parts of the ventricle do not remain active for the same length of time (Einthoven, 1913). Thus the region at which the stimuli are applied may have a longer or shorter refractory period than the region at which electrical measurements are made. When the electrogram of the whole heart is used this effect is exaggerated because the electrical activity begins with the reaction of the first region to be excited and ends with the recovery of the last region to be repolarized.

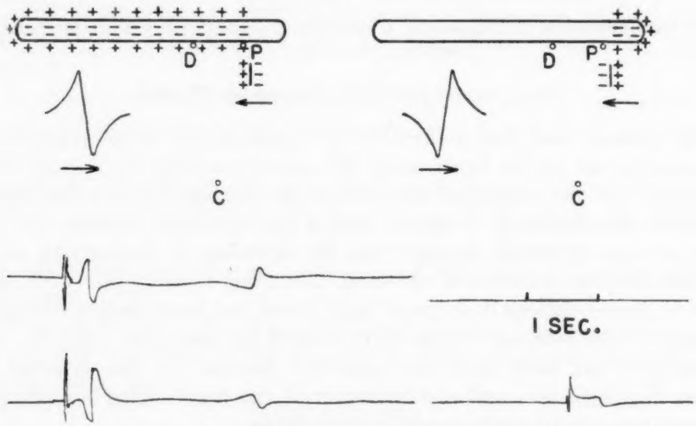

Fig. 1. The upper half of the figure illustrates the theory of the method described in the text. On the lower left is a record of the electrical changes from two neighboring points of a ventricular strip. From left to right the first complex is due to the electrical stimulus, the following interval is the conduction time to the recording electrode. The first diphasic wave is due to depolarization which is maintained for some time. Subsequent repolarization is indicated by the second diphasic wave. On the lower right is a similar record from a spontaneously beating auricular strip. The timing indicated applies to both records. The Q-T interval is measured from the point where the middle stroke of the depolarization wave crosses the base line to the similar point where the repolarization wave crosses the base line.

It appears that the only satisfactory way to solve this problem in heart tissue is to use a method which records the potential changes at one region and measures the refractory period simultaneously as near as possible to that region.

Experimental method. In the turtle heart experiments, strips of the ventricle or auricle, usually about 2 cm. in length and 2 or 3 mm. in width, were used. Except in the experiments in which contraction was studied, the strips were dissected at least 1 day and sometimes 2 or 3 days before using. For the purely electrical experiments they were set up on filter

paper on a paraffin block and kept irrigated with Ringer's solution. The strips were stimulated by means of two electrodes placed transversely at one end. Near these (see fig. 1) usually about 1 mm. distant, was one lead-off electrode P while another D was situated about 1 cm. distally. Both of these electrodes touched the strip while a third C was placed about equi-distant from P and D and about 5 cm. away. A separate amplifier with an ink recorder was connected to measure the potential differences occurring between each of the pairs of electrodes PC and DC. The pair PC recorded electrical variations near the stimulated region and the pair DC recorded the changes in the distal region if these were desired.

For the experiments in which contraction was studied simultaneously with electrical changes the muscle tension was recorded photographically using a glass rod as a lever of the so-called isometric type. In these experiments shorter strips, about 1 cm. long, were used to reduce the time of conduction. These strips were used within one to several hours after dissection. They were immersed in a small bath of Ringer's solution, but the distant electrode C (fig. 1) was still placed on a field of filter paper running out from the edge of the bath. In order to relate records of contraction and electrical changes which were taken simultaneously but recorded separately, the stimulus was recorded on each and each was accurately timed.

Outline of theory. The theory of the potential variations with the electrode arrangement used is illustrated in figure 1. If, as shown on the left side of this figure, a muscle bundle is stimulated between the electrode P and the right hand end, depolarization will occur at this end. The subsequent changes of potential as depolarization proceeds will be entirely equivalent to those produced by the passage along the bundle of the dipole shown underneath (Craib, 1927, 1930; Wilson, Macleod and Barker, 1933). The potential of the distant electrode C will remain approximately at the same value as that of the mid region of the dipole, which may be taken as zero. Thus P will be positive, zero, and negative, respectively, with respect to C as the dipole approaches it, is opposite to it, and recedes from it. A record of the potential of P with respect to C will, therefore, be like the diphasic curve drawn under the bundle. A record using electrodes D and C will have the same form but will occur later.

In a muscle bundle such as we used all of the fibers are depolarized and appear to remain in this condition for some time before repolarization commences. If the bundle recovers from the stimulated end the potential of P with respect to C will undergo, as illustrated on the right hand of figure 1 by an oppositely directed dipole, a series of changes similar to those occurring during depolarization but in the reverse order, P being first negative to C and later positive. If, on the other hand, recovery begins at the end last excited, i.e., on the left in the figure, the recovery will be in the same

order but will reach D first. Thus depolarization and repolarization will give rise to a record from either pair of electrodes PC or DC consisting of two diphasic waves separated by an interval which measures directly how long the depolarized condition lasted at P. It should be emphasized that the equivalence of the dipole to the partly polarized cell is in no wise altered by such external conditions as the amount of fluid surrounding the muscle or its conductivity, etc. These conditions will, however, determine the magnitude of the potential difference recorded between electrodes and also the proper position for the electrodes in the field. In the experiments to be described, it is implied that the muscle is surrounded by a homogeneous field and that the electrode C is essentially normal to the muscle both at P and D and that the front of the wave of depolarization is at right angles to the axis of the muscle bundle. These requirements, for various reasons, will usually be met sufficiently well although seldom exactly. It is implied also in figure 1 that in a given region both the depolarization and repolarization are abrupt rather than gradual processes. Which they are is not known for single cells, but heart strips repolarize more gradually than they depolarize as is evidenced by the fact that the amplitude of the recovery wave is different from that of the depolarization wave. This effect in bundles might be due to the cells being out of step in their abrupt recovery or it might be due to the recovery being progressive at each region of each cell. Because of this, some uncertainty is introduced in deducing the exact timing of the electrical events at a given point. This is not serious in the present work because the interval between depolarization and repolarization is much larger usually than the time required by either process.

Experimental results. *Type of record.* In figure 1 (lower left) is given a pair of records from a strip of turtle ventricle using electrodes such as PC and DC. It will be seen that there are two diphasic waves as expected but the recovery wave is small in amplitude denoting less abrupt repolarization than depolarization (see Wilson et al., loc. c., p. 37). Figure 1 (lower right) is from a spontaneously beating auricular strip. It may be said that in general the depolarized condition does not last nearly as long in the auricle as in the ventricle. Most of our records are of this general character although there are minor variations in form. In particular, either the depolarization or the repolarization wave may have one of its phases greater in amplitude than the other. There is seldom any doubt, however, about the choice of the point denoting the passing of the middle of the dipole, i.e., the point at which the wave swings across the base line from the one side to the other. It should be emphasized that we present curves of the expected form not to support the dipole theory outlined above but only to indicate that the experiments are adequate. Assuming the validity of the membrane hypothesis the dipole theory is a

direct deduction from electrical potential theory which is based in turn on experiments much more conclusive than living tissue is likely to provide. When the record is not in accord with the dipole theory it simply means that there are divergences from the experimental conditions postulated. The only question that can arise apart from experimental conditions is whether or not the muscle bundle acts sufficiently like a single uniform cell with the same orientation to enable one to use the dipole theory in interpreting the record. In our experience it does act sufficiently like such a cell, provided there are no newly cut or injured regions in the bundle.

Refractory period. To determine the refractory period the shocks were usually about five times the normal threshold strength. These will determine a period somewhat greater than the absolutely refractory period but only slightly so as the early relative refractory period shows very rapid recovery. Stronger shocks were avoided because of their probable effect on the repolarization process. While making these measurements the heart was driven at a constant rate and extra test shocks were placed nearer and nearer to the repolarization wave until no extra response occurred. After the extra shock gave rise to a response it was necessary to allow the Q-T interval to return to a value normal for the steady driving rate before testing again.

In table 1 are given several sets of data from ventricular strips. In some of these cases (in which the recording was done so close to the stimulating electrode that the Q wave and the shock were not separable) the time from the last normal shock to the extra shock is compared with the time from the last normal shock to the recovery wave. In other cases the Q wave was separate and the shock to extra shock interval was compared with Q-T. Both of these methods are essentially the same. Usually the time for the effective shock is taken as a mean of the times to a just-effective and to a just-ineffective shock. Since the extra shock distorts the T wave the normal shock to T interval is taken from the previous normal beat. It will be seen that the absolutely refractory period coincides with the shock to T interval quite closely except in the one set; in this the point of recording was not close to the point of stimulation. We conclude from these results that the tissue at a given region is absolutely refractory while it is depolarized. Different regions of the same strip may be depolarized for different lengths of time. Consequently both types of measurement must be made at the same region as nearly as possible in making a valid comparison. Failure always to respect this requirement may account for the disagreement between various workers.

Q-T interval and diastolic interval. While driving the heart at a regular slow rate the Q-T interval was studied as a function of the diastolic interval for a single interpolated shock. A typical set of data is shown in figure 2

in which the Q-T interval for the beat following a single early shock is plotted against the interval from the preceding T wave to this shock. This

TABLE 1

EXPERIMENT	SHOCK TO SHOCK	SHOCK TO T	EXPERIMENT	SHOCK TO SHOCK	Q-T
	seconds	*seconds*		*seconds*	*seconds*
1	2.38	2.28	3	2.24	2.16
	2.28	2.28		2.24	2.20
	2.32	2.28		2.07	2.00
	2.15	2.04 D		1.92	2.04
	2.02	2.04 D			
2	2.52	2.50	4	2.39	2.20
	2.08	2.12 D		2.14	2.00
	2.04	2.08 D		1.78	1.68 D
				1.86	1.70 D
			5	1.66	1.70
				1.74	1.80
				1.34	1.48
				1.44	1.68
				1.02	0.80

Several sets of data relating the Q-T interval to the absolutely refractory period. The one column gives the minimum interval from the usual to the test shock. The other column gives the interval from the usual shock to the T wave of the resulting response when the shock and Q are inseparable, otherwise it gives the Q-T interval. Each group of measurements is on a different tissue. Each pair was taken at different times. The results marked D were obtained after the Q-T interval had been shortened by digoxin. In experiments 1 to 4 the electrical measurements were made within 1 or 2 mm. of the stimulating electrode. In experiment 5 the electrogram was taken from a region about 1 cm. from the locus of stimulation.

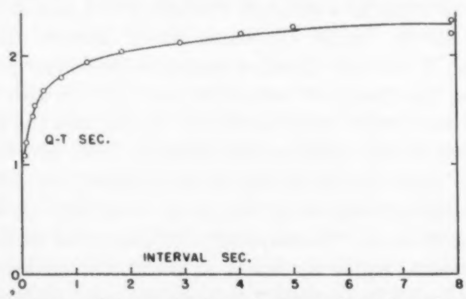

Fig. 2. The Q-T interval for a single early beat plotted against the interval between the last regular T wave and the shock eliciting the early beat.

relates the variation of the Q-T of any early beat with the earliness of the beat. In this particular experiment the intervals between the T waves

and the succeeding shocks during the steady driving were 8 seconds. A still slower rate would probably not bring about any greater lengthening of Q-T. A single extra shock put in after 5 seconds' interval changed the

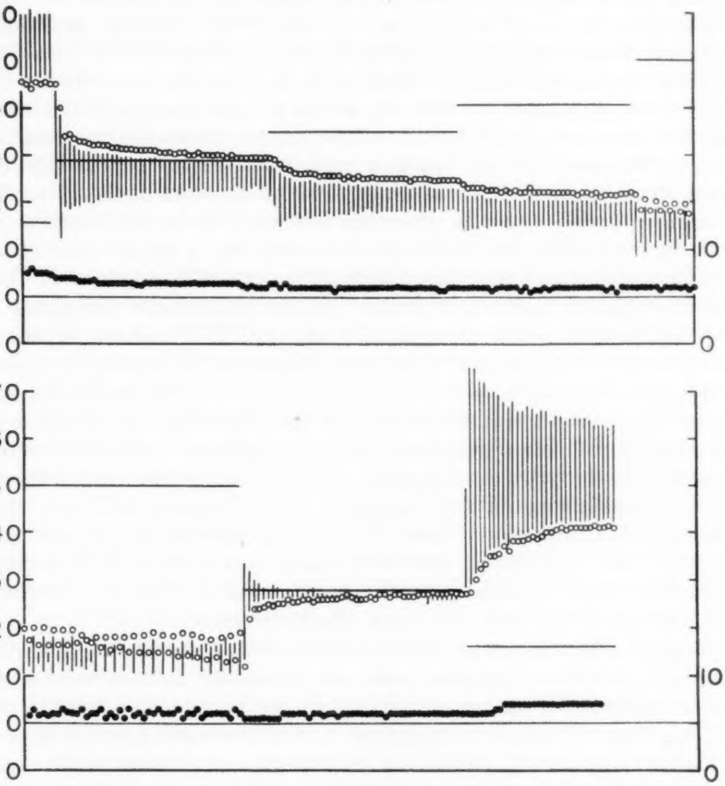

Fig. 3. On the upper ordinate scale are plotted on an arbitrary scale Q-T intervals, circles, and T-Q intervals, the ends of the vertical lines farthest from the circles. With each Q-T interval is plotted the succeeding T-Q interval. The driving shock lies within the T-Q interval. The rates in beats per minute are given, according to the ordinate scale, by the positions of the horizontal lines. The number of beats at any given rate can be found by counting the beats under these lines as each successive beat is plotted for the whole experiment. The dots plotted on the lower ordinate scale are conduction times between the shocks to the Q waves.

ensuing Q-T very little. But as shorter and shorter intervals were tried, marked shortening of Q-T occurred until finally an extra shock placed just after the T wave caused the ensuing Q-T to be only about half as long as the original. Thus the well known dependence of the Q-T interval

on heart rate is due not only to the cumulative effects of rate but to each disatolic interval as it occurs.

Q-T interval and heart rate. Cumulative rate effects do exist, however. In figure 3 are plotted Q-T intervals and T-Q intervals for successive beats during a series of increasing then decreasing rates. Starting at about 16 beats per minute and then changing the rate to 29 shortens Q-T markedly at first, in keeping with the shortening of T-Q, the phenomenon just considered. In addition, however, it will be seen that Q-T now slowly shortens to a new steady level. This process allows T-Q to lengthen, because the two intervals together must equal on the average the constant shock to shock interval which determines, in turn, the rate. The shortening of Q-T would be somewhat faster if T-Q did not lengthen.

On increasing the rate further it will be seen that a similar quick change and then drift of Q-T occurs each time until a rate of 50 is attained. Then the intervals are unable to stabilize. At the beginning of this phase the first T-Q is quite short, consequently the next Q-T is short; because of this the next T-Q is long and this in turn lengthens the next Q-T and so on. Ordinarily this reciprocal action produces only a small movement in or out of the T wave and stabilization is produced analogously to that determined by a mechanical governor. In the present case, however, the T-Q interval is of the order of 0.5 second. This value is at the bend in figure 2 where a small change in T-Q produces a large change in Q-T. In consequence of this there is an overshooting of each interval due to a change in the other and stabilization does not occur. Attempts to drive the heart strip under these conditions usually result sooner or later in a response being missed, and then a two to one rhythm obtains.

On decreasing the rate to 28 per minute it will be seen that Q-T interval lengthens appreciably at once and then gradually drifts toward a still greater value. On further slowing to 16 per minute, the original rate, Q-T no longer responds as promptly to the increased diastolic interval but moves continuously toward a greater length. At the end of the experiment it is still 10 per cent shorter than the original. Complete recovery might eventually occur but not necessarily because of the observed tendency for Q-T to shorten slightly, 5 per cent or 10 per cent per hour even with steady driving at 15 to 20 per minute.

Heart rate and conduction time. The interrelation of heart rate and conduction time was also studied in the foregoing experiment and is illustrated in figure 3. On the lower ordinate scale from 0 to 10 are plotted the relative times from the shock to the Q wave for each of the beats represented above. Each dot represents the conduction time for the beat succeeding the diastolic interval plotted directly above, i.e., for the beat whose Q-T interval is plotted in the next column. It will be observed that the expected shortening of conduction time with increase of rate is

usually obtained. There is some tendency during the period of irregular beating seen on the lower left of the figure for conduction to be faster after a short diastole and slower after a long diastole. Thus conduction may also be influenced by the diastolic interval per se as well by the rate of beating. Its dependency on diastolic interval is not always parallel to that of Q-T, however, because if the shock comes very early after the T wave the conduction then occurring during the relatively refractory period is very slow again. In addition it will be observed in the last two stages of the experiment that the slowing of conduction is considerably delayed and then is suddenly completed within one and three beats respectively. Such observations make it difficult to draw definite conclusions about the precise relation of conduction to either the diastolic interval or the rate of beating. It should be stated that the term conduction time as used above and later includes any electrical latent period there may be as well as true conduction time. Our observations lead to the conclusion that the latent period, if it exists at all, is a negligible part of the whole time.

Heart rate and Q-T interval in human subjects. In order to see whether the drifting of Q-T discussed above would occur in the human heart, several subjects were studied. The heart rate was changed by a short period of exercise. Figure 4 gives a typical example of the results. The beat intervals and the Q-T intervals are plotted here for successive beats, first for a short normal period before and then for several minutes after exercise. It will be seen that following exercise the heart rate is higher and the Q-T interval considerably shorter. When the heart rate has slowed to normal the Q-T interval is still short, but it continues to lengthen until it attains the value 40, about 10 per cent super normal. It retains this value at the end of the experiment even though the rate, after having been somewhat slow for a time, is again at the original level. It is suggested by these results that the Q-T interval may be a more sensitive index of the state of the muscle following exercise than is the heart rate. It is evident that the Q-T interval in the human heart is subject to influences other than those which are directly related to rate. Consequently there can be no exact general relation between heart rate and Q-T interval alone. Numerous formulae purporting to express such limited relationship have been submitted. It is obvious from the present observations, however, that such formulae must contain unexpressed relations concerning the equilibration of Q-T at each rate. It would be misleading, therefore, to consider them to be other than empirical relations of practical usefulness.

Q-T interval and contraction. In keeping with the idea that the Q-T interval or refractory period of cardiac tissue outlasts the rising phase of contraction so that tetanus cannot occur, it is stated that, for the heart in situ, the T wave usually coincides with the closure of the aortic valve.

However, attempts to show exact correspondences between electrical and mechanical events have led, on the whole, to the conclusion that they do not exist (Katz, 1928). This is to be expected, perhaps, because any definite relation between contractility and electrical activity can apply to the local

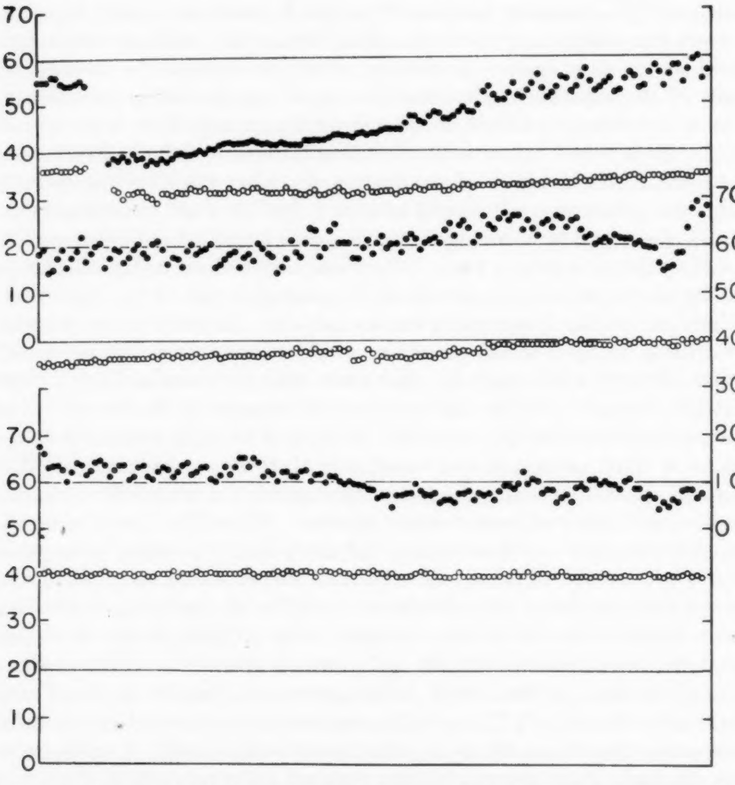

Fig. 4. The intervals between beats, Q-Q intervals, in dots plotted along with the included Q-T intervals in circles for a human subject preceding and following a short period of exercise indicated by the break near the top left. The data of successive beats are plotted before and after the exercise. The ordinate scale for the middle part of the figure is given on the right hand side. The ordinates are in arbitrary units.

region only. In the whole heart both electrical and mechanical events occur in an order determined by a conduction process, and the duration of activity at different regions is different. Consequently any local intrinsic relation is not only likely to be submerged in the activity of the whole

organ, but there is no great likelihood that two different hearts will integrate their activities in exactly the same way. For these reasons we deemed it advisable for the most part to seek a relation between Q-T and the mechanical events in short strips of tissue in which conduction time is relatively short. The essentials of the method were described above.

In figure 5 is a set of mechanical records of a ventricular strip driven at four different rates. It will be observed that the beats are markedly shortened in duration and decreased in height as the rate is increased.

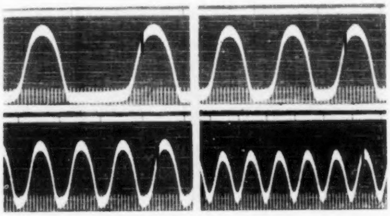

Fig. 5

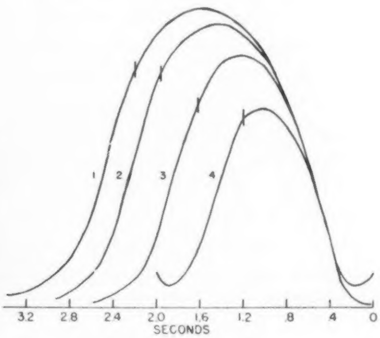

Fig. 6

Fig. 5. Isometric contractions of a strip of turtle ventricle at four different driving rates. The rising phase of contraction goes from right to left following the stimulus which is recorded by the break in the horizontal line above. The stroke on the falling phase of a representative curve of each class marks the time of occurrence of the T wave. This is taken from a record like figure 1 and is located by the stimulus which is common to both records. The interval between the vertical timing lines is 0.2 sec.

Fig. 6. The marked curves of figure 5 are plotted on the same scale so the early rising phases coincide. The two middle curves had to be moved, 0.04 sec. in the case of the larger and 0.06 in the case of the smaller toward the left in order to get coincidence. For these curves the shock would occur at 0.04 and 0.06 sec. respectively, for the others at zero time. The vertical strokes mark the times of the T wave. Relaxation was incomplete at the highest rate.

This is shown more clearly in figure 6 in which the curves are drawn so as to coincide during the rising phase. In order to accomplish this the two curves at the next to the lowest and next to highest rates respectively had to be shifted 0.04 and 0.06 second toward the left. These curves rose slightly earlier than the slowest, probably because conduction improved with increase in rate. The fastest curve matched the slowest without shifting. It should be emphasized that over a wide range of the rising phases one can choose a tension common to all the curves and hold that at this particular tension the rate of contraction is the same in each. This

similarity in the early stages of contraction leads directly to the conclusion that the contractile process begins unaffected by the change in rate of beating. Consequently the effect of rate is to modify the later stages of contraction only, and one is led to seek relations between the rate of beating and the duration of the contraction.

To study this the position of the T wave is marked on one curve of each set (fig. 6) by laying off on the mechanical record the shock to T interval of the corresponding electrical record. It will be seen that following the T wave relaxation is rapid in all curves and runs a similar course in numbers 1 to 3. Therefore, some event occurring about the time of the T wave reduces the muscle to the same state in all cases except that at the fastest rate. In that instance relative prolongation of conduction, compared to the duration of the beat, probably accounts for the lower relaxation. This relation of the T wave to relaxation suggests that the repolarization indicated by the T wave stops the contraction process. The alternative suggestion that preparation for rapid relaxation elicits repolarization is weakened by the fact already emphasized that the contraction in each case started in the same manner and presumably would have continued the same in the absence of interference. But if repolarization stops the contraction process, it is necessary to account for the fact that slow relaxation has been taking place for some time before the T wave is written. In other words, it must be shown that repolarization commences soon enough before the point where the T wave crosses the line in changing phase, which point is taken for the time of writing of T, to account for the initial decline of tension from the peak of the curve. With the present method of recording it does, and this is illustrated in figure 1. The T wave here begins to rise about 0.5 second before its reversal. The beginning of T must be due, however, to the existence of repolarization somewhere. Therefore, repolarization must have started at least 0.5 second before the middle of the wave. This time can be deduced more exactly as follows: preceding the T wave repolarization must have started at the first point to recover the sum of the time required for it to go to completion at that point and the time required for conduction from that point to the recording electrode. This repolarization time at a given point is the interval between the peaks of the diphasic T wave at that point. In figure 1, for example, this time is approximately 0.15 second while the conduction time for depolarization (shock to Q interval) is approximately 0.33 second. In this case, as can be seen from the two records together, the T wave is conducted at about the same rate as Q, and repolarization begins 0.48 second before T. For the data of figures 5 and 6 the corresponding time is 0.4 second. This estimate is only approximate because it assumes that recovery started at the same place as excitation and traveled at the same rate over the same path. None of these assumptions is necessarily valid,

and in fact it is rather unlikely that the T wave will always travel at the same rate as Q because, as is well known, it moves much more slowly in the intact ventricle. Therefore the time given, 0.4 second, is the least time that the beginning of repolarization can precede T.

Recording of T at the point of stimulation will not solve the difficulty for it cannot be safely assumed that recovery will necessarily begin at the first point excited. Moreover if it did, repolarization would spread evenly from the region of the contact electrode and a diphasic record would not be obtained. Furthermore, since the contraction cannot be localized to the point of electrical measurement, conduction effects are unavoidable. For these reasons we stimulated in the middle of 1 cm. strips and recorded electrically from about the middle of each end section. If, as was usually the case, the T wave was written about the same time from each half, repolarization probably started at the middle of the strip. Consequently the T wave indicated that about one-half of the strip had become repolarized.

As stated above, repolarization in the experiment of figure 5 started about 0.4 second before the T wave. Of this time 0.25 was required for conduction to the middle of each end section. A similar additional time would be required for complete repolarization to go the remainder of the way to the two ends. In considering the range of influence of the T wave, it may be said to extend backward about 0.4 second or 0.25 second depending on whether the beginning of, or the completion of repolarization, respectively, is the more important for relaxation. Applying these ideas to the curves of figure 5 the T wave follows the peak of the curve at the slowest rate by 0.6 second. Consequently in this case if repolarization stopped the contractile process it must have begun to do so at least this early. With the other curves their contraction can be said to be altered at the point at which they start to diverge from the slowest curve. Curve 2 ceases to coincide with 1 at 0.86 second before its T wave. Curve 3 diverges from 1, 0.74 second before its T wave while curve 4 diverges from 1 about 0.66 second before its T wave. These divergences occur considerably sooner than 0.4 second, the latest predicted beginning of repolarization. This is possibly due to the slower conduction of the T wave than of that of Q, which was the only one measured. This type of correlation is difficult to establish particularly in ventricular strips for a number of reasons. There is a source of error, for example, in this experiment in that a considerable number of beats were recorded at each rate, and in going from the slowest rate to highest successive curves may be lowered somewhat by fatigue. It will be seen from figure 6 that small decreases in height of the middle curves in particular would cause marked changes in their points of departure from the uppermost curve. An increase of conduction rate of T as well as Q in going from the second curve to the third

may account for the relatively earlier T wave in the latter. This will not account for the T wave of the fourth curve being relatively earlier than the third, but in this case the point of departure is not definite. We have found in other experiments with longer ventricular strips that conduction changes with rate of beating altered the contraction rate too much to make possible the type of comparison of the beats used above.

These possible sources of divergence should be reduced in the experiment recorded in figure 7 in which an auricular strip was used. In the auricle the conduction rate is much faster and the experiment was done so as to

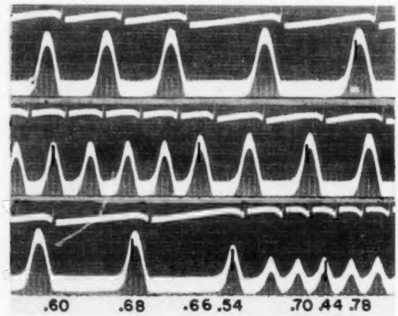

Fig. 7

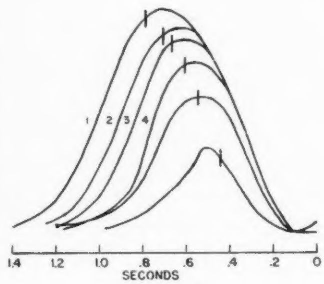

Fig. 8

Fig. 7. A series of contractions of an auricular strip driven at different rates. The records go from right to left. The stimulating shock is indicated by the beginning of the break in the upper line. The form of this line is not determined by the shape of the shock but by the capacity of the recording circuit. The upper two records are consecutive, a few beats are omitted between the second and third. The vertical lines mark the times of occurrence of the T wave in the several curves whose shock to T intervals are given numerically immediately below. The rate at the upper right and the lower left is 17.6. The fastest rate on the lower right is 63. The longest vertical lines mark 0.2 sec.

Fig. 8. The marked curves of figure 8 drawn to the same scale and placed so the shock is at zero time for all. The T wave is indicated by a vertical line in each curve.

cover the series of rates in a much shorter time. In figure 8 these data are plotted as in the previous experiment. Now, however, none of the curves are shifted to allow for conduction change. It will be seen that the four slowest curves 1 to 4 coincide in the initial portions indicating that here again the contraction starts off independent of rate of beating. The two fastest curves show, however, marked slowing of conduction. The influence of the T wave on contraction is again indicated by the points of divergence of the second, third and fourth curves from the first. In this experiment the intervals from these points to the T waves are 0.18 second, 0.19

second and 0.2 second respectively. These increase slightly as the rate increases, contrary to the results with the ventricle. This time 0.2 second corresponds to about the beginning of repolarization in the auricular strip, as deduced by the argument used above for the ventricle. Repolarization time, and conduction time in particular, are shorter here. This experiment which can be given more weight than the previous one on the ventricle, supports the view that the beginning of repolarization inhibits the contraction process.

It should also be noted in figure 7 that although the heart rates at the upper right and the lower left of the figure are the same the contractions are quite different while their shock to T intervals are different. The contraction at the lower left is very similar to that of the middle right hand two curves for which the rate is much faster. These latter sets of curves are similar while their shock to T intervals are similar. These results can be taken as further indication that the contraction is not controlled by the rate directly but through the effect of rate on the shock to T interval.

Another point to be noted in figures 6 and 8 is that the time of appearance of the T wave bears no relation to the extent of contraction. Such evidence is very much against the idea that repolarization is set off by the contraction process having reached a certain stage.

Further evidence that the polarization controls contraction is given by experiments with digoxin. This drug will markedly shorten the shock to T interval. At the same time the duration of the beat shortens even though the rate is maintained constant. These data will be presented at another time.

Another method, rather less direct, of relating Q-T and contraction leads to the same conclusions. The basis of this method is illustrated in figure 9. If the horizontal line here represents the beginning and duration of Q-T at the proximal region of the contracting strip, the upper line will serve the same purpose for a distal region, and intermediate lines could be drawn for intermediate regions. The conduction time of Q will be given by Q-Q and the conduction time of the recovery wave by T-T, made equal to Q-Q for simplicity. Contraction will begin just after the first Q, and its subsequent course will depend in part on the local properties of the muscle and in part on the conduction rate. The rise of tension may be represented by the curve on the lower left of figure 9. According to the hypothesis adduced above, relaxation will be initiated by repolarization, and its subsequent course will be determined by the conduction of the repolarization wave and the various mechanical properties of the muscle. The decline of tension may be represented by curve 1 on the right hand of figure 9. If now Q-T is shortened at all points to a new value Q-T_2 leaving conduction the same, it follows from the hypothesis that the new relaxa-

tion curve 2 will be of the same shape as 1 but earlier in occurrence by the same amount as T_2 is earlier than T_1. And for any further shortening of Q-T the same will be true providing conduction is essentially the same.

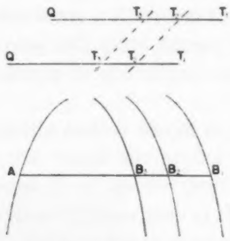

Fig. 9. Description in text

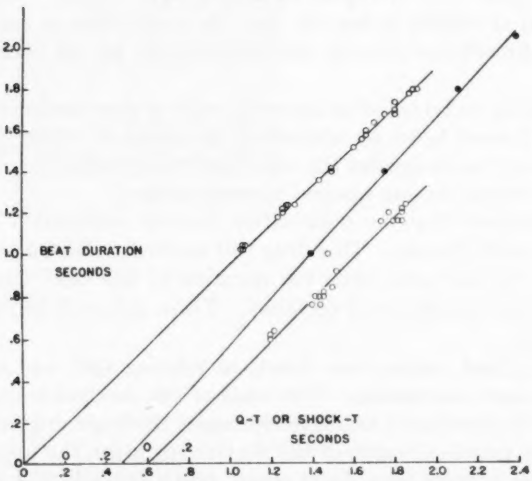

Fig. 10. The left hand and right hand curves are the Q-T intervals as abscissae and the widths in seconds of the contractions—the quantities AB of figure 9 as ordinates. These data are for consecutive beats of ventricular strips driven at increasing, then decreasing rates in experiments analogous to those of figure 7. The middle line is for the data of figure 5. In this case shock to T instead of Q-T is plotted. The width of the curves of figure 5 was measured at a level corresponding to about one-half the height of the lower right hand set of curves. The origins of abscissae for the three curves are given by the three 0 points in the same order from left to right.

Consequently any line AB measuring the width of the contraction in seconds at a given tension level will be shortened in proportion to Q-T.

A convenient way of testing this hypothesis is to plot the width AB against Q-T to see if a straight line is obtained. This is done in figure 10. It will be seen that the two sets of data plotted with Q-T intervals as ab-

scissae fall closely on straight lines going through the origin. Consequently the duration of contraction as measured here is directly proportional to Q-T and the hypothesis that Q-T controls contraction is substantiated further. The other curve in which shock to T is plotted is also linear, but, as is to be expected, it cuts the axis of abscissae at a point corresponding to the shock to Q interval. These data are therefore in agreement with the hypothesis also. The only widely diverging datum observed is the one point belonging to the curve on the right. In this case the beat is too wide for the Q-T. This beat followed a period of rapid beating after a pause of several seconds. It was considerably enhanced in height even over the normal at very slow rates. This exception may be due to alterations in conduction.

Conduction changes occurring equally for Q and T probably would not disturb the relation of Q-T to contraction because they would tend to alter the slopes of the rising and falling phases of figure 9 equally, leaving their separations essentially the same. Such changes occurred in the data of figure 10. If the conduction of Q and T are altered disproportionately, however, the beats concerned will no longer be comparable. The exceptional beat in figure 10 probably belongs to this class.

It will be seen from figure 9 that if the duration of the beat measured at the tension at which the line AB is drawn is proportional to Q-T this could not still be true if the duration of the beat were measured lower down. For a duration measured lower down the lines of figure 10 would still be straight, but they would not pass through the origin. The beat durations for the line going through the origin in figure 10 are approximately equal to the Q-T intervals. If Q starts contraction and T ends it, there should be some line AB for which A is the average time of beginning of contraction of the various regions of the muscle and B is the average time for relaxation, and this line AB should then be equal to the average value of Q-T. If all the Q-T intervals are equal any one is an average. Consequently, according to the hypothesis and as realized in figure 10, a beat duration at the proper tension level is the average contraction time for the muscle elements. Thus a definite meaning in conformity with the hypothesis can be given to the beat duration measured from about the middle of the rapidly rising phase of contraction.

Conclusions regarding Q-T interval and contraction. From these results we conclude that the contractile process, which is released by the depolarization wave consequent to excitation, proceeds as long as the membrane remains depolarized. When the membrane begins to repolarize at a given region the contractile process in that region is either retarded or arrested. If retarded only, it is almost certainly completely arrested by the time repolarization is complete. Thus polarization governs contraction, not contraction polarization, and the effects of rate are primarily on

polarization. It cannot be denied, that there is a third factor determined by the heart rate which simultaneously arrests contraction and starts repolarization. This indeed seems probable, but in the absence of any direct knowledge of the relation between excitatory and contractile processes there is no present ground for speculation on this point.

Relation to skeletal muscle. The above conclusions are quite different from those which can be drawn from skeletal muscle. With that tissue repolarization is complete in less than 1 millisecond, i.e., before contraction starts. It may be that contractile energy is released only while the membrane is depolarized and that its action persists for about 0.05 second after its release, this being the approximate duration of the rising phase of contraction. In the heart it may be true also that the contraction energy is not all expended as released, but may persist in its action for a similar time after repolarization commences. This time is too short, however, to have any discernible effect in the heart by present methods of observation.

Q-T and contraction in the intact heart. The lowest curve of figure 7 illustrates the difficulty which may be encountered when the conduction time is long either on account of low velocity or long distance. In this case repolarization follows so closely on depolarization that relaxation begins at the first point stimulated very soon after contraction begins. In consequence no great amount of the muscle is contracting at a given time, and the tension never gets very high. On the other hand, contraction exists somewhere for a relatively long time because the conduction is so slow. In the whole ventricle the conduction time for depolarization (Q-R-S interval) is relatively short, but the conduction time for repolarization (duration of the T wave) is long. Changes in the former will probably have only small effects on contraction under most conditions, but changes in the latter may be quite important. This possibility makes it unlikely that a definite relation between the T wave and contraction will hold for all heart rates. The inverted T wave may provide another difficulty. This is an index that the wave of repolarization travels in the same direction as the wave of depolarization instead of contrary to it. Since repolarization now proceeds from inside to outside of the ventricle relaxation of the muscle presumably proceeds in the same way. Consequently it may not be assumed without investigation that the inverted T wave will bear the same relation to contraction of the whole organ as does the erect T wave. On the whole, however, since the peak of the T wave probably indicates the time at which repolarization is proceeding in the bulk of the muscle, it is to be expected that relaxation of the bulk of the muscle will bear a fairly definite relation to it. The relation of the Q-T interval to the duration of the beat as evidenced in figure 10 contains much more promise for practical use than the position of the T wave alone. It is very probable that this relation will ordinarily hold for the whole heart.

SUMMARY

In turtle heart strips the electrical activity is recorded from pairs of electrodes one member of each pair being against the tissue and the other at a distance. The record permits the measurement of the interval between the depolarization and the repolarization of the tissue at a given region. This interval is called the Q-T interval. It is shown to coincide with the absolutely refractory period. It is shortened to one-half or less of its maximal value in a single very early beat. Further slow shortening occurs when the strip is driven for periods at a series of increasing rates. The lengthening of Q-T on slowing the rate is a slower process. Evidence is presented that repolarization arrests the contractile process in the muscle leading to the conclusion that the electrical processes control the mechanical rather than the mechanical, the electrical. In this connection it is shown that the duration of contraction as measured, for example, from half contraction to half relaxation is related linearly to the Q-T interval over a wide range. In the human heart it is shown that in recovery from exercise there is no fixed relation between the Q-T interval and the rate.

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THE EFFECT OF CHOLERESIS ON THE RATE OF EXCRETION OF INTRAVENOUSLY INJECTED BILIRUBIN

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This investigation was undertaken for several reasons. *First,* relatively little is known regarding the rate of excretion in the bile of intravenously injected bilirubin. However, considerable is known regarding its disappearance from the blood plasma of man and animals (1, 2) because bilirubin clearance is used as a liver function test (3, 4). The rate of removal of such a substance from the blood plasma does not mean that it is eliminated in the bile at the same rate. *Second,* the effect of choleresis produced by the intravenous injection of a bile salt on the rate of elimination of intravenously injected bilirubin in the bile has not been studied. *Third,* the effect of choleresis, similarly produced, on the rate of removal of bilirubin from the blood has not been adequately studied. Dragstedt and Mills (1, 5) found in a few experiments that the intravenous injection of sodium dehydrocholate had no effect or appeared to slightly augment the rate of removal of intravenously injected bilirubin, whereas in patients the injection of sodium dehydrocholate (Decholin-Sodium) has been reported to elevate (6) and to decrease serum bilirubin (7). *Fourth,* whether the administration of bile salts to patients after the release of a common duct obstruction or with retention of bile pigment due to other causes will increase the rate of excretion of the retained pigment is an open question. Sodium dehydrocholate, however, is used clinically for such a purpose, even though it is known that, under normal conditions in the dog, the production of a choleresis by bile salts does not increase the total daily output of bile pigments in the bile (8, 9). *Fifth,* since bile contains other pigments than bilirubin and *since the pure bilirubin prepared from bile for intravenous injection has different solubility properties from "bilirubin" in bile,* we have studied the rate of removal of pigment with and without choleresis when bile was injected intravenously.

Methods. Adult dogs weighing between 12 and 15 kilos were used. The animals were anesthetized with nembutal (sodium pentobarbital), using 64 mgm. of the drug per 5 lbs. The abdomen was opened, the common duct was cannulated for the collection of bile, and the cystic

duct was ligated. In 19 experiments *the kidney pedicles were ligated* tightly with linen thread so as to eliminate any possible loss through the urine (12). Pure indirect-reacting bilirubin obtained from Wilson Laboratories, Chicago, was dissolved in an alkaline solution (pH 9–10) and injected intravenously in doses of 5 and 12 mgm. per kilo. When the smaller dose was used, the bilirubin solution was injected into the femoral vein within one minute, whereas with the larger dose, five minutes were allowed for injection. Two concentrations of sodium dehydrocholate solution were used, a 1 per cent and a 10 per cent solution. They were injected into the blood stream through the femoral vein by means of a Woodyatt pump at a constant rate of 1 cc. per minute. Thus, when the 10 per cent solution of dehydrocholate was injected the animal received 6.0 grams per hour and when the 1 per cent solution was injected 0.6 gram per hour. This latter dose is more "physiological," since it represents the hourly output when the animal is producing 7.2 grams of cholates daily.

After the operation the bile was allowed to flow for approximately 1.5 to 2 hours until the volume and pigment output became constant. A half-hour control bile flow was collected and the bilirubin solution was injected and the bile collected every 0.5 hour for a total duration of 3 hours. Samples of blood were withdrawn at 15, 30, 60 minutes and in some cases at 180 minutes. The bilirubin determinations were made on the blood and bile by the Thannhauser and Anderson (10) modification of the Van den Bergh procedure. In the experiments in which whole dog bile was injected, the total pigment in the bile was determined by the method of Schmidt, Jones and Ivy (11) and the bilirubin content by the Thannhauser and Anderson method (10). Sterile gall bladder and hepatic bile were mixed and the total pigment, bilirubin and cholic acid content was determined before injection. The whole bile was injected intravenously into the femoral vein by means of a syringe at a very slow rate so that the total period of injection took 15 minutes. An amount of whole bile containing 5 mgm. of bilirubin per kilo was administered. The blood pressure varied considerably during the injection and immediately following; the dose was above the intravenous bile-salt tolerance of several dogs. The whole bile that was injected contained from 200 to 900 mgm. of cholic acid, and when 1 per cent Decholin was injected at a constant rate of 1 cc. per minute, 800 to 1500 mgm. of "cholic acid" were injected during the first hour.

RESULTS. *Injection of 5 mgm. per Kilo of Bilirubin; Kidney Pedicles not Ligated. Without 10 per cent sodium dehydrocholate.* The results of a control experiment on 5 dogs are shown in table 1A. In the column labeled "extra" output are the milligrams of pigment excreted during 3 hours in excess of the control output calculated for 3 hours. It is to be noted that the five dogs excreted 39.4 per cent of the injected bilirubin in 3 hours and 29 per cent in 1.5 hours. The maximum recovery occurred during the second half-hour period.

With 10 per cent sodium dehydrocholate. The results of injecting 5 mgm. per kilo of bilirubin during hydrocholeresis provoked by 10 per cent sodium dehydrocholate is shown in table 1B. It is to be noted that 37.7 per cent of the bilirubin was excreted in the bile in 3 hours and 29.4 per cent in 1.5 hours.

Comment. These results show that hydrocholeresis provoked by the injection of 10 per cent dehydrocholate solution did not alter the rate of

TABLE 1A

Intravenous injection of 5 mgm. per kilo of bilirubin (kidney pedicles not ligated)

REGIME	DOG NUMBERS	WEIGHTS	LENGTH OF EXPERIMENT	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RECOVERED	PER CENT RECOVERED IN 1½ HOURS	PER CENT RECOVERED DURING ½ hr.	1 hr.	1½ hr.
		kgm.	*hours*	*mgm.*	*mgm.*					
Control	1	17.0	3	84.0	33.89	40.3	32.0	9	14	9
	2	16.0	3	80.5	35.26	43.8	24.0	5	9	10
	3	14.0	3	70.5	26.35	37.3	30.0	5	15	10
	4	14.5	3	72.5	31.91	44.0	33.0	11	13	9
	5	13.6	3	68.0	20.84	30.6	25.0	9	9	7
Average.......	5	15	3	75.1	29.65	39.4	29.0	8	12	9

1B

Injection of 10 per cent decholin and 5 mgm. of bilirubin per kilo (kidney pedicles not ligated)

REGIME	DOG NUMBERS	WEIGHTS	LENGTH OF EXPERIMENT	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RECOVERED	PER CENT RECOVERED IN 1½ HOURS	PER CENT RECOVERED DURING ½ hr.	1 hr.	1½ hr.
10 per cent decholin (1 cc./min.)	1	14.3	3	71.5	26.25	36.8	32.0	12	13	7
	2	18.0	3	90.0	31.40	34.9	25.0	10	9	6
	3	12.3	3	61.0	20.39	33.4	33.0	14	10	9
	4	13.0	3	65.0	16.05	24.7	22.0	9	7	6
	5	21.3	3	106.0	51.08	48.2	33.0	11	12	10
	6	11.0	3	54.5	19.19	35.2	28.0	12	10	6
	7	13.2	3	66.0	32.03	48.5	37.0	12	14	11
	8	11.4	3	57.0	18.80	33.0	25.0	12	9	4
Average.......	8	14.3	3	71.4	26.89	37.7	29.4	12	10	7

Under anesthesia when 5 mgm. per kilo of bilirubin are injected, the plasma bilirubin is 2 mgm. per 100 cc. of plasma at the end of 1 hour.

excretion of the injected bilirubin. One difference was noted, however; the maximum excretion of bilirubin in the bile occurred in the second half-hour period when no bile salt was given and in the first half-hour period when the bile salt was given (table 1).

Since only about 38 per cent of the intravenously injected bilirubin was recovered in the bile in 3 hours, it was decided to ascertain the effect of removal of the kidneys on the excretion of bilirubin in the bile. Also, since none of these animals developed visible icterus and the plasma bilirubin

concentration at 1 hour is only about 2 mgm. per 100 cc. of plasma, it was decided to inject a larger quantity of bilirubin. So in the following experiments the kidney pedicles were ligated and 12 mgm. per kilo of bilirubin were injected. It was thought that with this higher plasma content of bilirubin, choleresis might have an effect on the rate of excretion of the pigment.

Injection of 12 mgm. per Kilo of Bilirubin; Kidney Pedicles Ligated. Without 10 per cent sodium dehydrocholate. In 9 dogs 12 mgm. per kilo of bilirubin were injected and no dehydrocholate was given. The results are shown in table 2A. About 36 per cent of the injected bilirubin was excreted in the bile in 3 hours and 25 per cent in 1.5 hours. The maximum excretion occurred in the second half-hour period as when 5 mgm. per kilo of the pigment was injected. At the end of 1 hour the plasma still contained 6.2 mgm. of the pigment per 100 cc. The animals did not become visibly icteric at 3 hours.

It is interesting that the per cent of bilirubin excreted was approximately the same as when 5 mgm. per kilo was injected.

With 10 per cent sodium dehydrocholate. In 5 dogs 12 mgm. per kilo of bilirubin were injected with 10 per cent dehydrocholate. The dehydrocholate reduced the rate of excretion of the injected bilirubin, since only 14.5 per cent was recovered in the bile in 3 hours and 9.0 per cent in 1.5 hours (table 2B). At the end of one hour the amount of bilirubin in the plasma was about the same as when dehydrocholate was not given.

All of these dogs developed visible icterus. Either the dehydrocholate affected the permeability of the capillaries, so that the *indirect*-reacting bilirubin injected passed into the tissues in sufficient quantity to stain the tissues, or some of the indirect bilirubin was changed to *direct*-reacting bilirubin which passed into the tissues. At any rate so much bilirubin entered the tissues that the excretion of the pigment in the bile was decreased 2.7 times during a 3 hour period.

Comment. When 10 per cent sodium dehydrocholate is injected at 1 cc. per minute the animals receive about 6 grams per hour. This is a large dose, perhaps large enough to injure the capillaries. A dose of sodium dehydrocholate was then selected which was considered to be more "physiological", or one (1 per cent solution) that approximates the average hourly 24 hour absorption of natural cholates from the intestine, namely, 0.6 gram per hour. This dose still caused a brisk choleresis.

With 1 per cent sodium dehydrochloate. One per cent sodium dehydrocholate were used and 12 mgm. per kilo of bilirubin were given. The results are shown in table 2C. In this experiment about 41 per cent of the bilirubin was excreted in the bile in 3 hours and 24 per cent in 1.5 hours. Thus, choleresis again did not significantly augment the rate of excretion of bilirubin. Though the bilirubin concentration in the plasma was about

the same as with 10 per cent dehydrocholate administration, no visible jaundice occurred.

TABLE 2A

Intravenous injection of 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

REGIME	DOG NUMBERS	WEIGHTS	LENGTH OF EXPERIMENT	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RECOVERED	PER CENT RECOVERED 1½ HOURS	PER CENT RECOVERED DURING			BLOOD PLASMA BILIRUBIN MGM./100 CC.		
								½ hr.	1 hr.	1½ hr.	15	30	60 min.
		kgm.	hours	mgm.	mgm.								
Control	1	8.0	3	96.0	36.01	37.5	26	4	13	9			
	2	18.0	3	216.0	65.52	30.3	18	1	9	8			
	3	18.0	3	216.0	64.33	30.0	18	1	8	9			
	4	14.0	3	168.0	60.66	36.1	27	2	15	10			
	5	11.0	3	132.0	44.34	33.5	21	3	11	7	10.0	6.2	5.1
	6	12.0	3	144.0	43.02	30.0	18	2	9	7	11.1	10.0	6.2
	7	10.0	3	120.0	45.07	37.5	22	1	11	10	10.4	10.0	9.8
	8	9.0	3	108.0	34.82	30.7	19	7	5	7	8.8	8.3	6.1
	9	10.0	3	120.0	52.99	44.1	27	9	9	9	10.7	7.9	4.1
Average	9	12.0	3	146.6	49.64	33.8	21.8	3	10	9	10.2	8.5	6.2

2B

Injection of 10 per cent decholin and 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

10 per cent decholin (1 cc./min.)	1	14.0	3	165	30.08	18.0	10	2	4	4			
	2	14.0	3	168	26.34	15.7	11	3	4	4			
	3	13.0	3	156	15.51	9.9	8	2	3	3			
	4	13.0	3	156	14.46	9.3	8	2	3	3	10.0	9.1	7.9
	5	17.0	3	204	37.49	18.3	11	3	4	4	10.0	8.8	6.9
Average	5	14.0	3	170.0	24.78	14.5	9.0	2	4	3	10.0	8.9	7.4

2C

Injection of 1 per cent decholin and 12 mgm. of bilirubin per kilo (kidney pedicles ligated)

1 per cent decholin (1 cc./min.)	1	18.0	3	216	98.00	45.3	30	11	10	9	12.5	11.1	7.5
	2	10.0	3	120	51.32	42.7	23	0	13	10	8.3	7.0	5.4
	3	8.0	3	96	27.00	28.1	16	5	6	5	8.1	7.5	6.1
	4	11.0	3	132	54.53	41.3	25	7	10	8	9.3		7.0
	5	12.0	3	144	63.76	44.2	28	10	9	9	13.0	10.7	9.3
Average	5	11.8	3	141.6	58.92	41.6	24.4	6	10	8	10.2	9.0	7.0

Comment. Obviously in these experiments the dose of sodium dehydrocholate determined the amount of the injected pigment which was excreted in the bile. With 10 per cent dehydrocholate the tissues became visibly stained and less pigment was excreted in the bile. With 1 per cent de-

dehydrocholate the tissues did not become visibly stained and about the same amount of pigment was excreted in the bile as in the controls which received no bile salt. However, the amount of pigment is also a factor in determining the development of icterus because 10 per cent dehydrocholate did not cause visible icterus of the tissues when 5 instead of 12 mgm. per kilo of bilirubin were injected. Since visible icterus of the tissues was present or absent with approximately the same plasma levels of bilirubin 1 hour after injection, it is possible that, when the plasma bilirubin was high at the start as with a 12 mgm. injection and the concentration of dehydrocholate was also high (10 per cent solution), some of the pigment was converted into the more diffusible direct-reacting pigment. This would account for the visible icterus and the decreased excretion of pigment in the bile when 12 mgm. per kilo of pigment were injected in association with 10 per cent sodium dehydrocholate. However, it seems more probable that some ratio between indirect-reacting bilirubin and capillary permeability or a threshold exists.

The Rate of Excretion of Pigment Injected in the Form of Whole Dog Bile; Kidney Pedicles Ligated. Since the solubility of bilirubin isolated from bile by chemical procedures is different from the solubility of bilirubin in bile, it was considered important to determine the rate of excretion of the pigment as it occurs in bile. Because there are other pigments in bile than bilirubin, both the total pigment and bilirubin were determined.

Without 1 per cent sodium dehydrocholate. In 5 dogs 5 mgm. of bilirubin per kilo body weight of dog gall bladder bile and hepatic bile were injected. The results are shown in table 3A and 3C. Approximately 9 per cent and 11 per cent of the bilirubin and total pigment respectively were recovered during 3 hours, and in each case 6 per cent was recovered during the first 1.5 hours. In all cases bilirubin was present in the plasma after 3 hours and averaged 3.2 mgm. per 100 cc. of plasma.

With 1 per cent sodium dehydrocholate. In 5 dogs 1 per cent sodium dehydrocholate was injected with whole dog bile. About 10 per cent of the bilirubin and 12 per cent of the total pigment was recovered in 3 hours and 7 per cent in 1.5 hours (table 3B and 3D). At the end of 3 hours the plasma bilirubin was approximately 2.4 mgm. per 100 cc., which was about the same as when dehydrocholate was not given.

Comment. When whole dog bile and 1 per cent sodium dehydrocholate were injected, the amount of bilirubin recovered was about the same as that recovered when 12 mgm. of bilirubin and 10 per cent dehydrocholate were injected, i.e., 10 per cent and 14.5 per cent respectively. No visible icterus developed in any of the dogs in which whole dog bile and dehydrocholate were injected. The direct-reacting bilirubin in the bile probably diffused into the tissues but the concentration of pigment was not great enough to cause visible icterus of the tissues. If 12 mgm. of pigment per kilo had

TABLE 3A

Intravenous injection of 5 mgm. per kilo of bilirubin as contained in whole dog bile

REGIME	DOG NUMBERS	WEIGHTS	LENGTH OF EXPERIMENT	CHOLIC ACID IN DOG BILE	INTAKE	"EXTRA" OUTPUT	TOTAL PER CENT RECOVERED	PER CENT RECOVERED 1.5 HOURS	PER CENT RECOVERED DURING ½ hr.	1 hr.	1½ hr.	BLOOD PLASMA BILIRUBIN CONC., MGM./100 CC. 15	30	60	180 min.
		kgm.	*hours*	*mgm.*	*mgm.*	*mgm.*									
Control	1	9	3	820	43.7	3.98	9	4	1	1	2	5.9	7.1		4.4
	2	10	3	902	50.6	3.47	7	4	1	0	1	9.3	9.7	6.0	3.4
	3	9	3	309	44.95	3.77	8	6	4	1	1		9.7	6.5	3.0
	4	7	3	245	34.88	3.81	11	8	1	4	3	8.4	8.7	5.0	2.6
	5	8	3	450	39.95	4.07	10	7	2	3	2	9.8	8.7	5.0	2.4
Average	5	8.6	3	545	42.81	3.82	9	6	2	2	2	8.2	8.8	5.6	3.2

3B

Intravenous injection of 5 mgm. per kilo of bilirubin (whole dog bile) and 1 per cent decholin

1 per cent	1	9	3	309	44.95	6.19	13	8	3	3	2				
decholin	2	9	3	309	44.95	6.77	15	9	4	3	2	8.7	8.2	5.0	2.4
(1 cc./	3	8	3	600	40.00	2.49	6	3	0	2	1	9.0	8.5	5.4	3.0
min.)	4	11	3	690	54.81	3.61	6	2	0	1	1	8.7	8.0	5.4	2.4
	5	8	3	510	39.95	3.87	9	6	2	3	1	9.0	8.4	4.4	2.0
Average	5	9	3	483	44.93	4.59	10	6	2	3	1	8.8	8.3	5.0	2.4

3C

Recovery in the bile of "total pigment" after intravenous injection of dog bile

Control	1	9	3	820	107.2	12.42	12	4	1	1	2				
	2	10	3	902	117.9	12.74	6	2	0	1	1				
	3	9	3	309	112.3	21.10	18	13	7	2	3				
	4	7	3	245	87.2	6.12	7	4	2	1	1				
	5	8	3	450	98.4	8.45	8	5	2	2	1				
Average	5	8.6	3	545	104.6	12.16	11	6	3	1	2				

3D

Recovery in the bile of "total pigment" after intravenous injection of dog bile and 1 per cent decholin

1 per cent	1	9	3	309	112.3	16.05	14	9	3	4	2				
decholin	2	9	3	309	112.3	17.80	16	9	3	3	3				
(1 cc./	3	8	3	600	120.0	15.65	13	8	3	3	2				
min.)	4	11	3	690	164.4	14.57	9	6	2	3	1				
	5	8	3	510	120.0	13.60	11	8	3	4	1				
Average	5	9	3	483	125.8	15.53	12	8	3	3	2				

been injected instead of 5 mgm., visible icterus probably would have resulted.

Because of the toxicity of whole bile this experiment is more pathological than physiological in type. However, if it is essential to use a bilirubin with the same solubility as that in bile, bile must be used, at least for the present.

Bilirubin Accounted for at the End of One Hour. The total pigment accounted for at the end of 1 hour may be calculated by adding that recovered in the bile to that still present in the blood plasma. The remainder must be in the cells or in the body fluids other than plasma in those experiments in which the kidneys were excluded. When 5 mgm. per kilo of bilirubin were injected without kidney exclusion and dehydrocholate, 34 per cent of the pigment was in the bile and blood plasma at one hour; when the same amount was injected with 10 per cent dehydrocholate 37 per cent was in the bile and blood plasma. When 12 mgm. per kilo of bilirubin were injected with kidney exclusion but without dehydrocholate 39 per cent was in the bile and blood plasma; under similar conditions but with 1 per cent dehydrocholate, 39 per cent of the pigment was in the bile and blood plasma, but with 10 per cent dehydrocholate only 30 per cent was in the bile and blood plasma. Since the latter dogs developed jaundice the difference between 39 and 30 per cent, or about 15 mgm. of pigment, represented a sufficient difference to cause visible tinting of the tissue.

It was noted that after a 3-hour period the rate of excretion was quite low regardless of the fact that choleresis during the third hour was the same as during the preceding hours. This shows that the rate of excretion of the bilirubin remaining in the body occurs at a slow rate. The time for complete removal will have to be determined on dogs with a permanent bile fistula.

General discussion. On the basis of the data obtained it is clear that choleresis induced by the intravenous injection of sodium dehydrocholate, a pure and relatively non-toxic choleretic agent, does not augment significantly the rate of excretion of intravenously injected bilirubin in the anesthetized dog. This result, however, does not necessarily indicate that the administration of sodium dehydrocholate, or a similarly acting choleretic, after the relief of common-duct obstruction, will not increase the rate of excretion of bilirubin. This question can only be answered directly by performing a controlled series of experiments on a group of animals in which jaundice is produced by obstruction of the common bile duct and then the obstruction released.

SUMMARY

Using anesthetized dogs with exclusion of the gall bladder and the common duct cannulated for the collection of bile, it was found that the

production of choleresis by the continuous intravenous injection of sodium dehydrocholate (0.6 or 6.0 grams per hour) does not increase the rate of removal of intravenously injected bilirubin (5 or 12 mgm. per kilo body weight) from the blood stream or its excretion in the bile. This is also true when whole dog bile is used as a source of bile pigment. When 12 mgm. of bilirubin per kilo body weight are injected, or the bilirubin level of the blood is elevated to 9 to 13 mgm. per 100 cc. of plasma, the injection of an excessive amount of sodium dehydrocholate (6.0 grams per hour in our experiments) converts a hyperbilirubinemia without jaundice into a hyperbilirubinemia with jaundice. These results do not necessarily indicate that the administration of sodium dehydrocholate, or a similarly acting choleretic, after the relief of common-duct obstruction will not increase the rate of excretion of bilirubin and the disappearance of jaundice.

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THE EFFECTS OF ESTROGEN AND THYROIDECTOMY IN FEMALE RATS ON THE EXCRETION OF CREATINE AND CREATININE[1]

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Among the various factors influencing creatinine and creatine excretion in mammals, the species, sex, age, diet and the hormonal state of the body are of outstanding importance. Both adult male and female rats normally excrete creatine when fed on diets containing creatine or creatine precursors, such as those which include meat scrap. Injection of thyrotropic hormone or thyroxin increases the excretion of creatine in rats (Pugsley, Anderson and Collip, 1934). On the other hand, thyroidectomy decreases and subsequent injections of thyroxin increase the excretion of creatine in male rats on a diet containing creatine or creatine precursors (Allison, Glaser and Leonard, 1939). Kun and Peczenik (1935) report that in female rats during diestrum or after castration, the creatine excretion is either low or lacking, whereas during estrum or after the administration of estrogens, the creatine excretion rises markedly. Beard and Jacob (1940b) report that castration in the male and female rat is followed by a temporary creatinuria (due to traumatic injury?) and that injection of sex hormones (estrogen, male hormone) induces creatinuria in recently castrated rats, but not in those deprived of their gonads for 90 days. The present investigation was undertaken to determine in female rats, fed a diet producing a marked creatinuria, 1, the effect of the estrous cycle; 2, of castration and thyroidectomy, and 3, of estrogen injections on the excretion of creatinine and creatine.

Materials and methods. The rats used in these experiments were young adult females (approximately 200 grams) of the Long-Evans or Langley strains. The piebald rats were raised on Purina dog chow, but throughout these experiments were fed Russell's diet BMS (1932), which is a modified Sherman diet B containing meat scrap. The Langley rats

[1] The work reported here was done under the sponsorship of the Bureau of Biological Research, Rutgers University and with the assistance of General Scientific Project, Division of Professional and Service Projects, Work Projects Administration of New Jersey.

were raised on diet BMS. They were placed in individual metabolism cages several days before urine collections were started to acclimate them to their new surroundings. Water and food were provided ad lib. The 24-hour urine samples, free from feces and food particles, were collected under toluene. The screen bottom of each cage and collecting funnel were washed with distilled water. The washings were combined with the urine sample and the volume made up to 100 ml. The body weights of the rats were measured at the time of the urine collection. The micromethods of Folin (1914) were used for the determinations of creatinine and creatine, autoclaving to convert the latter substance into creatinine. The results are expressed in millimols of creatinine or of creatine excreted in 24 hours per kilogram of body weight.

RESULTS. *Normal excretion.* Four female rats of the piebald strain (170–177 grams) were placed in the metabolism cages for a period of one month and occasional determinations of creatinine and creatine were made (15–24 hr. samples). For the next 27 days, daily determinations were made and the estrous cycles were followed by the vaginal smear method. These data are illustrated by circles in figure 1, where millimols of creatine are plotted against millimols of creatinine per kilogram of body weight. The averages for these 164 determinations, with the probable errors, are 0.302 $\pm$ 0.0023 millimol of creatinine and 0.163 $\pm$ 0.0032 millimol of creatine. At the end of these experiments the rats had gained an average of 32 grams. The millimols of creatinine excreted are distributed around the mean, which is independent of the magnitude of the excretion of creatine.

No correlation was found between the stages of the estrous cycle and the amount of excretion of creatine or of creatinine. The daily ratios of creatine to creatinine are plotted in figure 2 against the days of collection of the urine. The periods of heat occurred some time during the 24 hours represented by circles with crossed lines. The ratios were used, instead of absolute values, to illustrate variation in the excretion of creatine because they are not affected by small losses in urine which might occur during the collection of 24 hour samples. These data demonstrate that there is no marked effect of the period of heat upon these ratios.

Castration. These rats were castrated and after a period of 15 days daily determinations were made of creatine and creatinine for 17 days. These data are illustrated in figure 1 by the closed squares. The averages of the 68 determinations, with the probable errors, are 0.295 $\pm$ 0.0037 millimol of creatinine and 0.100 $\pm$ 0.0043 millimol of creatine per kilogram of body weight. There is no change from normal in the excretion of creatinine, but there is a drop in the excretion of creatine in these castrated females. For a statistical comparison, nine consecutive daily determinations of creatine in normal and castrated female rats were selected. Based

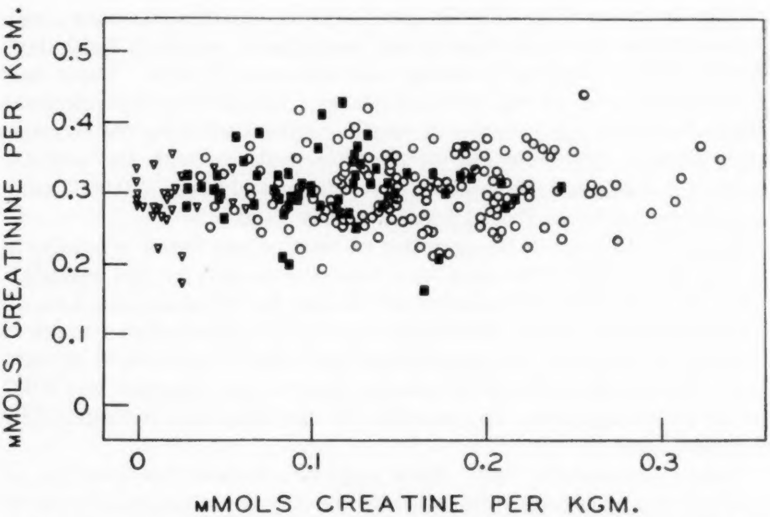

Fig. 1. The millimols of creatine excreted per kilogram of body weight during the 24 hours by four female rats, are plotted against the millimols of creatinine per kilogram excreted over the same period of time. The circles represent excretion in the normal, the closed squares excretion in the castrated and inverted triangles the excretion in the castrated, thyroidectomized female rats.

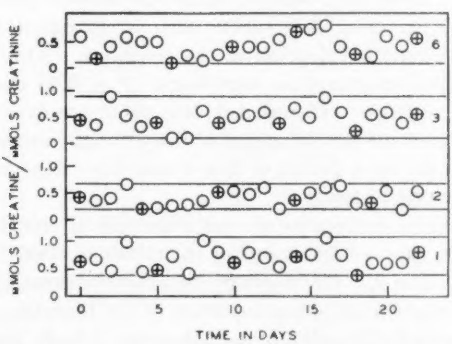

Fig. 2. The ratios between the millimols of creatine and creatinine excreted, are plotted against time in days. The data for rats 1, 2, 3 and 6 are plotted separately and the circles with crossed lines represent the ratios obtained during the 24 hour period when the rats were in heat.

on millimols of creatine excreted per kilogram of body weight, the drop in the excretion of creatine in castrated female rats is significant since P = 0.05 by Fischer's method.

Thyroidectomy. The thyroid glands (thyro-parathyroidectomy) were removed from the four piebald rats and after a period of eight days, determinations were made during nine consecutive days. These data illustrated in figure 1 by the inverted triangles, indicate that thyroidectomy markedly lowers the excretion of creatine without affecting the excretion of creatinine. The averages for 34 determinations, with the probable errors, are 0.291 ± 0.004 millimol of creatinine and 0.022 ± 0.0002 millimol of creatine excreted per kilogram of body weight.

A similar decrease in the excretion of creatine was found in females of the Langley strain, when they were thyroidectomized but not castrated. The mean excretion of millimols of creatine per kilogram based on 15 determinations on three normal rats was 0.109. Four other females of the same strain were thyroidectomized and after a rest period of eight days, the average millimol of creatine excreted per kilogram was 0.007 for 20 determinations. The excretion of creatinine was not altered by thyroidectomy.

Estrogen in castrated rats. Since castration lowered the excretion of creatine, it seemed important to study the effects of estrogenic hormone treatment in the castrated rats. The four castrated piebald rats, before they were thyroidectomized, were given a single injection of 60 R.U. of an estrogenic hormone (progynon B)[2]. The average excretion of creatinine and of creatine for five days preceding the injections are compared in table 1 with the average excretion of these substances for the five days following the injections. These data show a small but definite rise (0.105 to 0.134 millimol) in the excretion of creatine with no change in the excretion of creatinine following the injection of the hormone. This rise of creatine following estrogen treatment is significant ($P < 0.05$ and almost equal 0.02, Fischer's method). Included in this table are average millimols of creatinine and creatine per kilogram of body weight excreted by the four normal female rats for a period of five consecutive days taken at random from data appearing in figure 1.

The average daily excretions of creatinine and of creatine in four other castrated female rats before and after injections of 5000 R.U. of estrogen are recorded in table 2. The average excretions of creatinine and creatine during the first eight days before injection of the hormone are respectively 0.271 millimol and 0.073 millimol per kilogram of body weight. The rats were injected with 5000 R.U. of estrogen on the ninth day. The average millimols of creatine excreted per kilogram of body weight increased on the eleventh day coincidentally with the appearance of full heat in the vaginal smear. From the data presented in table 2, the average excretion of creatinine is 0.258 millimol and of creatine is 0.150 millimol per kilogram

[2] We express our thanks to Dr. W. R. Bond of the Schering Corporation, Bloomfield, N. J., for supplying us with progynon B.

during the six days the rats were in heat. The slightly lower average excretion of millimols of creatinine during the heat period is due to the

TABLE 1

Average millimols of creatinine and creatine per kilogram of body weight excreted daily for five days by four female rats under various experimental conditions

CONDITIONS	WEIGHT	CREATININE	CREATINE
	kgm.	*mmols/kgm.*	*mmols/kgm.*
Normal female	0.205	0.301	0.168
Castrated female	0.252	0.305	0.105
Castrated + estrogen (60 R.U.)	0.242	0.294	0.134
Castrated + thyroidectomy	0.247	0.295	0.024
Castrated + thyroidectomy + estrogen (60 R.U.)	0.251	0.290	0.027
Castrated + thyroidectomy + estrogen (20,000 R.U.)	0.247	0.290	0.023

TABLE 2

Average millimols of creatinine and creatine per kilogram of body weight excreted for eight days by four castrated female rats before and after the injection of 5000 R.U. of estrogen

DAYS	WEIGHT	CREATININE	CREATINE
	kgm.	*mmols/kgm.*	*mmols/kgm.*
1	0.214	0.269	0.099
2	0.219	0.240	0.067
3	0.222	0.293	0.097
4	0.225	0.301	0.044
5	0.234	0.293	0.050
6	0.237	0.278	0.078
7	0.240	0.212	0.032
8	0.240	0.266	0.089
9*	0.245	0.308	0.064
10	0.235	0.257	0.083
11	0.235	0.184	0.140
12	0.227	0.255	0.176
13	0.228	0.242	0.164
14	0.226	0.300	0.145
15	0.227	0.281	0.141
16	0.226	0.286	0.135

* Injected 5000 R.U. of estrogen.

marked drop in the excretion of this substance on the eleventh day, the significance of which is not clear.

Estrogen in thyroidectomized rats. The four piebald rats, which were both thyroidectomized and castrated, were injected with 60 R.U. of the

estrogenic hormone and after a period of five days received another injection of 20,000 R.U. The results are presented in table 1. The average millimols of creatine excreted per kilogram for five consecutive days preceding the injections are 0.024, after the first injections of 60 R.U. are 0.029 and after the massive dose are 0.023. The administration of the hormone, therefore, failed to influence the creatine level of excretion. The creatinine was unchanged.

The four rats of the Langley strain, which were thyroidectomized but not castrated, were injected with 5000 R.U. of estrogen. The average excretion of creatine on the day of injection was 0.0343 millimol and the average excretion for four days following the injection was 0.030 millimol. The excretion of creatinine was not altered by the injection. These experiments with thyroidectomized rats indicate that estrogen increases the excretion of creatine only when the thyroid glands are present.

Discussion. There was no apparent relationship between the excretion of creatine and creatinine in normal female rats exhibiting marked creatinuria. Since Block and Schoenheimer (1939), in their studies of the metabolic relationships of creatine and creatinine using isotopic nitrogen, found that body creatine is the precursor for the creatinine excreted in the urine, some relationship between the excretion of these two substances might be expected. Beard and Jacob (1940a), on the other hand, found that injection of creatine into rats did not increase the excretion of creatinine. Saturation of the body with creatine due to excess creatine or precursors in the diet of rats would explain the independence of this excretion of creatine and creatinine.

Contrary to the results of Kun and Peczenik (1935), the estrous cycle in our female rats could not be correlated with the daily fluctuations in the excretion of creatine. These fluctuations were as great in the castrated rats as in the normal rats, although at lower levels. Possibly the variations in the circulating concentration of estrogens in rats during the estrous cycle were not large enough under the conditions of our experiments to enable an effect on creatine excretion to be observed. Removal of the ovaries, for example, lowered the excretion of creatine slightly, but not to the extent reported by Kun and Peczenik. That there may be a sex difference in the effect of hypogonad function on creatinuria in the rat is suggested by the work of Coffman and Koch (1940), who state that "castration of adult male rats does not alter creatine excretion."

A marked reduction in the excretion of creatine in the female rat, as in the male rat (Allison, Glaser and Leonard, 1939), followed removal of the thyroid gland. The average excretion of creatinine by the thyroidectomized female rats remained within the range shown by the normal and castrated animals.

The increase in creatine excretion following injection of estrogen into

rats with intact thyroids is in agreement with the results of Kun and Peczenik (1935) and Pizzolato and Beard (1939). Since this response was not observed in the thyroidectomized animals, the thyroid gland may be an important intermediary organ in controlling the effect of estrogen on creatinuria. There are other evidences in the literature which support this suggestion. Anderson and Kennedy (1933), for example, have shown that gonadectomy produces atrophy of the thyroid in the mature female rat, which is most obvious eight weeks after the operation. The tendency for a lowering of the excretion of creatine, as well as the observations made by Beard and Jacob (1940b), that injection of sex hormones into 90-day castrated rats would not produce creatinuria, may be due to the atrophy of the thyroid.

There is also some evidence that the action of estrogen on thyroid activity, hence alterations in creatinuria, may occur by way of the hypophysis. Turner and Cupp (1940) have shown that gonadectomy lowers the thyrotropic hormone content of the hypophysis and that injections of estrogen maintain the normal level of thyrotropin in the castrated female hypophysis. Since it is known that thyrotropic hormone causes an increase in creatine excretion (Pugsley et al., 1934), it might be assumed that a decrease in the output of this hormone following castration would result in a lowered excretion of creatine. Furthermore, the administration of estrogens into castrated rats did not raise the level of excretion of creatine above normal in our experiments nor did they increase the thyrotropic hormone content of the hypophysis above normal in the experiments of Turner and Cupp. It is possible, therefore, that changes in creatine excretion, which result from female sex hormone imbalance of the body, may be due to alteration in thyroid function as mediated by the hypophysis.

SUMMARY

The data presented demonstrate 1, that the average excretion of creatinine remains constant in these normal, castrated, and thyroidectomized female rats, fed on a diet containing meat scraps; 2, that there is no apparent correlation between the estrous cycle and the excretion of creatine; 3, that castration lowers slightly the excretion of creatine; 4, that thyroidectomy lowers this excretion markedly, and 5, that estrogen increases the excretion of creatine in castrated rats, but has no effect upon the excretion of creatine in thyroidectomized rats.

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THE METABOLISM OF HUMAN SPERMATOZOA[1]

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In a preliminary communication (8) the metabolism of human spermatozoa in Ringer-glucose was briefly described. It was shown that the metabolism was almost exclusively glycolytic. The present report will deal in greater detail with the metabolic behavior of these cells.

The available information on the metabolism of human spermatozoa is scanty, and in the main is confined to data derived from incubation of semen (3, 6, 7). Recently, Shettles measured respiration and respiratory quotients using undiluted semen. Since Shettles' paper raises fundamental questions of technique, discussion of his results will be considered later.

Before presenting the present results, it is necessary to mention certain points about the use of Ringer-glucose as a medium. Since most measurements of tissue metabolism are made in an artificial medium such as Ringer's solution, it was considered desirable for purposes of comparison, to obtain such data for the spermatozoa. Furthermore, the use of Ringer-glucose has certain obvious advantages in that when lactic acid production in the presence of bicarbonate is measured, the CO_2 of glycolysis can be determined directly since Ringer's is a non-retentive medium. On the other hand, there are often objections to the use of artificial media, and as is well known tissues bathed in blood serum may show a greater and longer sustained metabolic activity than the same tissues in phosphate or Ringer's (15, 9). These authors have emphasized the technical difficulties inherent in the use of serum, these being, principally, the problems of measuring respiration *directly* in the presence of the serum bicarbonate and of allowing for the high CO_2 retention which is a characteristic of blood serum. One is faced with the same problems in the choice of a physiological medium for spermatozoa. Seminal fluid contains bicarbonate in about the same concentration as blood serum (3) so that direct measurements of O_2 consumption in the presence of KOH cannot be made. Furthermore, seminal fluid, like blood serum, "binds" CO_2 to a marked degree so that the manometric measurements of the CO_2 liberated from bicarbonate by

[1] Aided by a grant from the National Committee on Maternal Health.

lactic acid cannot be made without the use of CO_2 retention curves and other technical refinements.

As a final consideration in the choice of Ringer-glucose, the viability of the cells is of prime importance. Spermatozoa, considered as a tissue, are unique in that motility may be used as a criterion of viability. In all of these experiments, we have seldom met with an instance in which the motility of the cells after many hours in Ringer-glucose at 38°C. showed any decrease compared with the motility of the same cells in seminal fluid under the same experimental conditions. These points will be considered in greater detail under separate headings in the body of the paper.

Methods. The technique used was that of Warburg (14), and all results obtained manometrically except in a few cases where chemical determinations of lactic acid were made to check the manometric results.[2] The spermatozoa were obtained from young, healthy adults at intervals of four or five days and were received in the laboratory within an hour after ejaculation. The semen was then centrifuged, the seminal fluid decanted, and the cells transferred to Ringer-glucose. After the number of cells per cubic centimeter was determined and motility examined, the cells were transferred to the manometer vessels. Respiration was measured in phosphate (pH 7.4) by the direct method of Warburg (14) and in a bicarbonate medium by the use of the Summerson differential manometer (13). For measurement of glycolysis, bicarbonate was added to make a final concentration of 0.03M and the system equilibrated with gas mixtures containing 95 per cent 0_2 and 5 per cent CO_2 and 95 per cent N_2 and 5 per cent CO_2. All measurements were made in duplicate and manometric readings made every 30 minutes. The experimental periods ranged from 3 to 10 hours. The results given below represent the mean metabolism per hour during the first 3 hours.

Observations. I. *Glycolysis.* Since glycolysis is the outstanding feature (8) of the metabolism of human spermatozoa, this will be considered first. Table 1 gives figures for aerobic and anaerobic lactic acid formation. The results are expressed as mm.^{3} $CO_2/10^8$ cells/hour and, in more conventional fashion, as Q_G or the amount of CO_2 per mgm. of dry tissue per hour.[3]

The glycolysis figures are presented in two groups (table 1), the first representing the metabolism of 83 individual specimens and the second, 71

[2] In the latter experiments, the method of Friedman, Cotonio, and Shaffer (11) was used and these chemical determinations were found to check the manometric reading within ±8 per cent. I am indebted to Dr. S. B. Barker for making these determinations.

[3] Previously, these results were presented as mm$^3CO_2/10^6$ cells/hour. It was thought advisable here to use the higher number of cells so as to allow the use of whole numbers.

experiments in which two or more specimens were combined. The two groups are not combined since the standard error of the difference between the means shows that the glycolysis of the combined specimens is significantly higher than that of the individual specimens. The 83 experiments on individuals represent material from only 30 men, with either one or several specimens from each individual.

Table 2 represents figures obtained from the spermatozoa of 5 individuals at different times. It will be seen that while there are striking differences

TABLE 1

Mean anaerobic and aerobic glycolysis of individual and combined specimens

NUMBER OF EXPERIMENTS	ANAEROBIC GLYCOLYSIS					AEROBIC GLYCOLYSIS				
	mm.^{3} $CO_2/10^8$ cells/hour	High	Low	σ	Mean $Q_G^{N_2}$	mm.^{3} $CO_2/10^8$ cells/hour	High	Low	σ	Mean $Q_G^{O_2}$
Individual 83...	10.6	27.2	3.5	4.8	7.4	8.5	22.8	3.2	4.0	5.9
Combined 71 ...	12.1	24.6	3.2	3.9	8.4	10.4	22.0	3.2	3.9	7.2

TABLE 2

Anaerobic and aerobic glycolysis of individual specimens

DATE	NO. 1E MM.^{3} $CO_2/10^8$ CELLS/HOUR		DATE	NO. 2C MM.^{3} $CO_2/10^8$ CELLS/HOUR		DATE	NO. 3L MM.^{3} $CO_2/10^8$ CELLS/HOUR		DATE	NO. 4K MM.^{3} $CO_2/10^8$ CELLS/HOURS		DATE	NO. 5T MM.^{3} $CO_2/10^8$ CELLS/HOUR	
	N_2	O_2		N_2	O_2		N_2	O_2		N_2	O_2		N_2	O_2
2/24/39	12.1	10.7	3/27/39	7.1	6.8	2/15/40	24.5	22.8	4/ 5/39	15.9	15.9	3/31/39	4.9	5.0
3/14/39	7.4	7.2	3/31/39	8.1	7.0	2/19 40	24.3	21.6	4/ 8/39	11.2	10.0	4/ 7/39	4.9	4.0
3/29/39	10.0	8.5	4/ 5/39	6.6	6.6	2/23/40	27.2	22.0	4/18/39	10.3	8.0	4/14/39	3.5	3.2
4/ 5/39	11.0	9.2	4/11/39	13.2	12.5	2/29/40	20.6	19.0	4/26/39	13.4	12.6	4/28/39	5.6	4.0
4/26/39	8.5	7.9	4/19/39	7.5	7.0	3/ 4/40	28.0	20.0	4/29/39	11.7	9.0	5/31/39	10.0	8.0
6/ 6/39	11.4	11.4	4/25/39	10.1	9.6				5/24/39	9.7	7.3	9/22/39	7.7	6.0
			6/ 2/39	7.5	7.5									
			6/22/39	8.5	7.6									

in lactic acid production between individuals, the acid production of the sperm from any one remains relatively constant from week to week. Sharp rises or falls may occur from time to time which cannot be accounted for on the basis of motility since all of the specimens showed maximal and sustained motility. Whether the glycolysis is high or low, the ratio between aerobic and anaerobic glycolysis remains relatively constant, though in several instances there was no evident difference between aerobic and anaerobic metabolism.

There are striking differences in the glycolysis of spermatozoa of different

individuals. This is most apparent if the figures for 3L and 5T are compared. The acid production of 3L is about four times that of 5T and it is obvious that the glycolysis of one does not tend to approach that of the other even when one is high and the other low. Again, it should be emphasized that motility is not a factor as the sperm of both individuals showed maximal motility at all times.[4] In regard to cell morphology this factor can be dismissed briefly; no significant difference was found in any specimen throughout the course of these experiments. There is no evidence at present to explain the quantitative metabolic differences between specimens

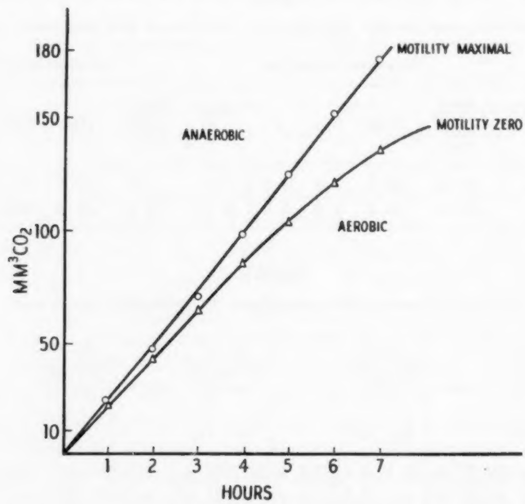

Fig. 1. Anaerobic and aerobic glycolysis of human spermatozoa in Ringer-glucose-bicarbonate. Number of cells present = 270 million. The motilities represented in the figure were taken at the end of the 7-hour period.

of different individuals and those which may be found from time to time in the same individual.

The relatively small difference between anaerobic and aerobic glycolysis (15–20 per cent) is striking when one considers that aerobic glycolysis, when present in normal adult tissues, is usually of small magnitude and never approaches the magnitude of the anaerobic metabolism.

Figure 1 shows an experiment in which aerobic and anaerobic glycolysis was measured on specimen 2C over a period of seven hours. This experiment is selected because it demonstrates attributes common to many

[4] In a study of sheep spermatozoa, Comstock (1) has found significant differences in glycolysis between semen of different rams, these differences being independent of motility and sperm numbers.

specimens of human spermatozoa. Anaerobic glycolysis is linear over the entire period while aerobic glycolysis tends to fall off slightly with time. This decrease in *aerobic* glycolysis becomes apparent, in most specimens, only after several hours, and is coincidental with a failure of motility. Human spermatozoa, under certain experimental conditions, are *sensitive to injury* in the presence of O_2 as evidenced by failure of motility and of lactic acid production. Figure 1 is a striking example of this phenomenon. At the end of the 7 hour experimental period, under identical conditions, motility in N_2 was maximal whereas motility in O_2 was zero. This effect in O_2 does not appear in every experiment because few of the experiments reported here lasted longer than 5 hours and a time factor is involved. In other words, the "O_2 effect" is not always an "all or none" phenomenon. Usually, it appears after the cells have been in air or O_2 for several hours at 38°C. If the motility in N_2 and O_2 be compared, no qualitative difference may be seen, but if the per cent of cells motile be calculated, the number of cells motile in O_2 is frequently sharply decreased. Thus, unless anticipated, this phenomenon may escape notice.

II. *Respiration.* The initial experiments in this research were designed to determine the minimal number of cells necessary for accurate determination of O_2 consumption and glycolysis. Concentrations of cells ranging from 50 to 300 million cells per cubic centimeter in Ringer-glucose-phosphate were set up in separate manometers for measurement of O_2 consumption by the direct method (2), 1 cc. of suspension being added to the vessels. Small vessels (8 cc. vol.) were used to obtain maximal sensitivity. Within this cell-concentration range, the O_2 consumption over a 3 hour period was so small as to appear negligible, particularly since the same cells in Ringer-glucose-bicarbonate showed an easily detectable anaerobic and aerobic lactic acid production. Thus, it was apparent that sufficient cells were present in the system to show an adequate O_2 consumption if that type of metabolism existed to any marked degree.

Since the respiration evidently was quite small and since large amounts of tissue were not available, it became necessary to work with a manometric system of greater sensitivity. The experiments were re-designed so that the 8 cc. vessels would be filled with the sperm suspension, leaving a gas phase of approximately 1.5 cc. and so increasing the sensitivity of the system about four times. It is obvious that KOH cannot readily be used in such a system for the absorption of respiratory CO_2, but since the fluid volume is large and the solubility of CO_2 is great relative to that of O_2, little CO_2 will escape into the gas phase. Under these conditions a small O_2 consumption was detectable in 25 experiments and the mean figure for the first hour is given in table 3. In 8 other experiments, no oxygen consumption was evident, although in each case the aerobic glycolysis was less than the anaerobic. Several measurements of oxygen

consumption were made in a bicarbonate medium using the differential manometer technique of Summerson (13). Continuous measurements are not possible with this technique, but the *total* O_2 consumption in bicarbonate is similar to that in phosphate. Because of the limited amount of spermatozoa available at any given time, and the exceedingly small respiration of these cells, the measurements of oxygen consumption presented here are subject to greater error than those of glycolysis.

In a series of experiments to determine if the O_2 consumption was cyanide sensitive, the results show that cyanide (10^{-3}M) does not inhibit O_2 consumption when the latter is detectable. Furthermore, motility is not depressed either in the presence of cyanide or of carbon monoxide. When these facts are considered with the maintenance of maximal motility under anaerobic conditions, it will be seen that the motile activity of human spermatozoa is not dependent on energy derived from respiration.

As Keilen (5) and others have shown, all actively respiring tissues, when examined spectroscopically under anaerobic conditions, show the absorption spectrum of reduced cytochrome. Spectroscopic examination of a

TABLE 3

Mean oxygen consumption per hour of individual specimens

NUMBER OF EXPERIMENTS	MEAN OXYGEN CONSUMPTION PER 10^8 CELLS PER HOUR	HIGH	LOW	MEAN Q_{O_2}
25	1.8	3	0.3	1.25

concentrated suspension of human spermatozoa shows that if cytochrome is present in these cells, it does not exist to any marked degree. No bands of reduced cytochrome can be seen.[5]

The only other figures available on the respiration of *human* spermatozoa are those given by Shettles (12) who measured the respiration and R.Q. in *semen*. His figures are about 7 times higher than the highest figures reported here, and it is rather difficult to account for such a large discrepancy solely on the basis of the cells being in seminal fluid in the one experiment and in Ringer-glucose in the other. However, on the basis of the use of untreated semen, Shettles' experiments are open to question on several counts. He used the *direct* method of Warburg in measuring respiration and the *first* method of Dickens and Simer in measuring respiratory quotients. These methods are described in detail by Dixon (2). As Dixon points out, these methods give reliable results only when the medium is buffered with phosphate and are subject to severe limitations when bicarbonate is present in the medium. Seminal fluid contains large

[5] I am indebted to Dr. Dean Burk for the use of his spectroscopic equipment and for much helpful advice in certain of the techniques used in this work.

amounts of bicarbonate and when, as in the direct method of Warburg it cannot be equilibrated with CO_2, the pH of the system shifts progressively to the alkaline side due to loss of CO_2. The measurement of respiratory quotients by the method used by Shettles is liable to serious inaccuracies because the presence of bicarbonate gives a value for preformed CO_2 which is high relative to respiratory CO_2.

However, we have repeated Shettles' experiments on the respiration of undiluted semen and are unable to confirm his results. He states that the O_2 consumption is proportional to the number of cells present and that seminal fluid devoid of spermatozoa had no O_2 consumption. We have used specimens of semen containing from 0.5 to 151 million sperm and specimens containing none. Table 4 shows the O_2 consumption of these specimens and the average O_2 consumed per 10^8 cells per hour. It will

TABLE 4

Oxygen consumption of undiluted semen

NUMBER OF CELLS IN SEMEN	OXYGEN CONSUMED PER HALF-HOUR (CU. MM.)						AVERAGE OXYGEN CONSUMED PER HOUR	AVERAGE OXYGEN CONSUMED PER 10^8 CELLS PER HOUR
	30	60	90	120	150	180		
millions								
0*	35	4.8	10	14			31.9	
0.5	34	4	3.6				26	52
3.5	14	4.8	2.4				11.6	3.3
28	40	18	8.4	8.4	6	6	28.9	1.0
70.5	31	2.4					16.7	0.23
75.5	40	2.4					21.2	0.28
131	24	12	9.6	4.8	4.8	4.8	20	1.52

* High leucocyte count.

be seen that in every case, during the first 30 minutes, there is an O_2 consumption which is high relative to that during successive 30 minute readings but that the overall O_2 consumption is in no way proportional to the number of cells present. It is likely that absorption of O_2 by the seminal fluid itself is a factor which has not properly been taken into account. The mechanism of such an absorption is not clear but it may be pointed out that cell-free blood serum also absorbs small amounts of O_2 under similar conditions (10).

III. *Substrates.* Added substrate in the form of utilizable sugar is essential for maintenance of glycolysis and of motility. Maltose, mannose, fructose and glycogen can be substituted for glucose with no evident difference in the level of glycolysis or the quality of motility, but the cells cannot utilize lactose, sucrose, or galactose. When these sugars are substituted for glucose, glycolysis and motility fail rapidly. The effective concentration of utilizable sugar lies between 20 and 200 mgm. per cent.

At high sugar levels (0.6–1.0 per cent) the spermatozoa show signs of toxicity as evidenced by a failure of motility.

IV. *The presence of bacteria.* Early in this work it became apparent that most specimens of human spermatozoa contained bacteria and that they were infected at the source, e.g., in the prostatic secretions or in the seminal vesicles. The presence of bacteria in the sperm suspensions at 38°C. usually is not manifested until between the third and sixth hours. Bacterial growth in the system shows itself by an increase in lactic acid production which proceeds logarithmically. The most persistent bacterium is a Gram-positive anaerobic streptococcus and the metabolic figures affected are those of anaerobic glycolysis. On occasion, the growth of aerobic bacteria is seen in a rapid rise in O_2 consumption. Though the kinetics of bacterial metabolism cannot be discussed at length in this paper, it can be shown that the metabolic figures for the spermatozoa are not affected by the presence of bacteria during the early hours of the experiments. This can be demonstrated briefly. We were fortunate in obtaining 3 donors whose specimens at no time showed bacterial contamination. The glycolysis figures for the sperm of these donors are represented in table 2 (2C, 1F and 5T). The figures for 2C and 1F fall within the normal metabolic range while those of 5T, on several occasions, were considerably lower. Since no bacterial growth was evident in any of these cases it is clear (*a*), that the mean figures for glycolysis of sterile specimens is within the limit of variation of specimens which are infected (see table 1), and (*b*), that wide variations in lactic acid production may exist between bacteria-free specimens of different individuals. Furthermore, it may be pointed out that the ratio between aerobic and anaerobic glycolysis during the first 3 hours in all cases is relatively constant. One would expect to find this ratio extremely unstable if bacteria contributed much acid during the early hours since bacterial growth may vary from time to time, particularly in the anaerobic system. But this ratio is quite constant during the first 3 hours.

DISCUSSION. The predominance of glycolysis and the relative deficiency of respiration in the metabolism of human spermatozoa, coupled with the fact that these cells retain maximal motility under anaerobic conditions would indicate that respiration is secondary and not an essential part of the metabolic function. Redenz (11) and Ivanow (4) come to similar conclusions for the sperm of the dog, ox and guinea pig in spite of the fact that these cells have a high respiration relative to the human. Comstock (1) also found that the respiratory activity of sheep spermatozoa is not associated with motility. In the work reported here, the *nature* of the O_2 consumption of human spermatozoa is left open for further investigation. It is not at all certain that the small O_2 consumption is operating through a cytochrome system, or indeed, that these cells possess, to any

degree, any of the hemin systems now known to be essential components of the main respiratory pathway in living cells. Analyses of the respiratory and glycolytic pathways of human spermatozoa are somewhat handicapped by the small amounts of tissue available at any given time, but investigations are in progress and will be reported later.

SUMMARY

1. The metabolism of human spermatozoa in Ringer-glucose is characterized by a high aerobic glycolysis and a relative deficiency in respiration.

2. The glycolysis of the spermatozoa of any one individual is relatively constant from specimen to specimen but large differences are found from individual to individual. These differences cannot be accounted for on the basis of motility.

3. Motility is not dependent upon the presence of oxygen, but added substrate in the form of utilizable sugar is essential for prolonged activity.

4. Prolonged exposure to air or to oxygen may result in failure of motility and of glycolysis.

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THE ABSORPTION OF CAROTENE FROM ISOLATED INTESTINAL LOOPS[1]

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Recently it has been shown that disturbances in the absorption of fat-soluble vitamins or their precursors can exist in humans suffering from biliary insufficiency and can lead to serious clinical manifestations of avitaminosis in spite of an adequate supply of these vitamins in the diet (2, 3, 5, 18). In view of the clinical importance of the intestinal absorption of fat-soluble vitamins and of the incomplete knowledge regarding the rôle of bile in this process, further study appeared to be desirable. The ease and accuracy with which carotene may be determined recommended it for this study.

Greaves and Schmidt (13, 14) were the first to demonstrate that bile acids are necessary for the absorption of carotene by rats. Their criterion for the absorption of carotene was the restoration of a normal vaginal smear picture in vitamin A deficient rats. Therefore, their results were only qualitative or roughly quantitative. Other investigators have demonstrated that a certain amount of normal absorption of fat is necessary for transportation of carotene across the intestinal wall. On a fat-free diet, rats assimilate only a small fraction of the amount that is completely utilized when 10 per cent fat is included in the diet (1). Clausen (6) has studied rates of absorption of carotene by determination of this provitamin in blood plasma.

In the present study the absorption of carotene has been investigated by the use of dogs with isolated intestinal loops. Known quantities of carotene were placed in the loops under a variety of conditions and the amount of absorption was determined by carotene analyses of the contents of the intestinal loop remaining after a period of absorption. This technique is advantageous for a number of reasons. It permits direct com-

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parison of the absorption in a large series of experiments on the same animal under a variety of conditions thus allowing a more accurate statistical analysis of the results. The use of such loops eliminates errors due to variations in the effective absorbing surface which would be unavoidable in the intact animal. In addition, determinations of carotene remaining in the loops after absorption periods would seem to be a more accurate means of estimating the amount of absorption than biological tests or than plasma analyses for carotene. Analysis of plasma for carotenoids provides a measure of the difference between the rate of absorption of carotene from the intestine and the rate of its absorption and utilization by the tissues and other body fluids rather than a direct index of intestinal absorption alone. Such plasma carotene determinations are valuable but do not provide a means for the quantitative evaluation of the effectiveness of various substances on the absorption of this provitamin. The use of intestinal loops obviates such difficulties.

Experimental. *Analytical method.* For the determination of carotene in the stock solutions and in the contents of the intestinal loops, the solution is saponified with 20 cc. of a freshly prepared solution of KOH in alcohol for each gram of sample. The saponification flasks are fitted with reflux condensers, and the contents are boiled on a steam bath for 30 minutes, or until saponification is complete. A stream of nitrogen is bubbled through the solution during the saponification period to prevent oxidation of carotene. The contents of the flask are then cooled, 50 to 100 cc. of ethyl ether are added, and after shaking for a minute or so and allowing to settle, the ether-alcohol mixture is decanted into a separatory funnel. This is repeated two additional times with small amounts of ether; then the residue in the flask, which sometimes forms an adherent mass, is broken up by shaking with a small quantity of 95 per cent ethyl alcohol. After two or three additional extractions with ether, the ether extract usually comes off colorless, and the residue is then discarded. About 250 cc. of ether are usually required for complete extraction. Next about 100 cc. of distilled water are poured gently through the alcohol-ether solution in the separatory funnel. The alkaline alcoholic-aqueous solution which separates is withdrawn from the bottom of the funnel and is re-extracted by shaking with ethyl ether in another separatory funnel. If an emulsion is encountered, it may be cleared by adding a little ethyl alcohol. The ether extracts are combined and washed with distilled water until free from alkali. Washing three or four times by pouring the water through the ether solution and down the sides of the funnel removes most of the alkali. The remainder is removed by gently shaking the ether solution with small quantities of water until the wash water no longer gives a color with phenolphthalein.

The ether solution containing carotene is dehydrated by permitting it to stand over anhydrous sodium sulfate for several hours. Then the ether

solution is decanted and the sodium sulfate is washed several times with anhydrous ether to remove any adsorbed carotene. The combined extracts are evaporated under a stream of nitrogen in an all-glass distillation apparatus. The final stage of evaporation to complete dryness is carried out under reduced pressure. The carotene residue in the flask is dissolved in petroleum ether (30–60° boiling fraction), the solution is made up to an appropriate volume in a volumetric flask, and readings are made in an Evelyn photoelectric colorimeter (11) using filter number 440 (transmission limits 410 to 475 mμ. Mean transmission at 440 mμ). Carotene is estimated from these galvanometer readings by reference to a standard calibration curve obtained by reading known dilutions of a sample of pure crystalline β-carotene. This standardization curve is shown in figure 1.

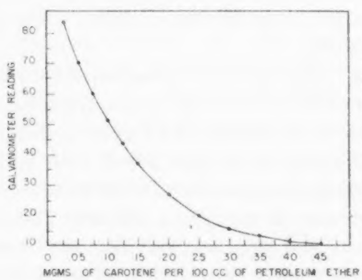

Fig. 1

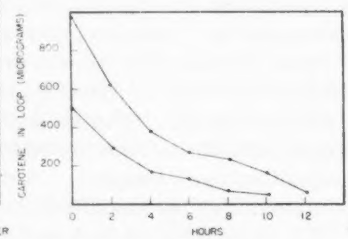

Fig. 2

Fig. 1. Standardization curve of β-carotene in petroleum ether. Percentage transmission at 440 mμ.

Fig. 2. Carotene absorption curves. Effect of carotene concentration on the rate of absorption in the presence of bile and pancreatic lipase.

Kimble (17) has used a photoelectric colorimeter for the determination of carotene in plasma. According to Kimble's procedure, the carotene extracted from the plasma is dissolved in petroleum ether for the photoelectric colorimeter readings. She has found that the color of carotene dissolved in petroleum ether does not follow Beer's law perfectly. Our results verify that conclusion. However, the L values conform fairly well in the middle region of the curve, and the determinations are quite satisfactory when dilutions are made so that the galvanometer readings fall along this portion of the standardization curve.

The results of analyses of standard stock solutions of carotene in cottonseed oil are presented in table 1. It may be seen in line one, column five, that the analyses yield an average of 99.1 per cent theoretical with a probable error of $\pm$ 2.1 per cent. Analyses of mixtures of known quantities of carotene in oil with lipase and bile are presented (line three). The

accuracy is of the same order. The accuracy also is not affected by digestion of these mixtures for six hours at 37°C. (line four).

Preparation of loops and technique of experiment. The loops were prepared by the technique described by Johnston (16). Only loops of the upper jejunum were used. Carotene was introduced into the loops in the form of saturated solutions of crystalline β-carotene in cotton-seed oil. Johnston's (16) double balloon technique was used for closing the loops, and the solutions were injected by means of the central catheter tube. After the introduction of the oil solutions, the tube was washed down with two 5 cc. portions of distilled water. A check on the accuracy of the entire technique was obtained by a number of experiments on the recovery of carotene placed in the intestinal loop and then withdrawn immediately

TABLE 1

Accuracy of method for determination of carotene in intestinal contents

TYPE OF ANALYSIS	CAROTENE THEORETICAL	NUMBER OF DETERMINATIONS	CAROTENE DETERMINED (AVERAGE)	CAROTENE RECOVERED
	micrograms		*micrograms*	*per cent*
Carotene in oil (1 cc. samples)	490	14	486	99.1 ±2.1
Carotene in oil (5 cc. samples)	2450	17	2421	98.9 ±2.3
Carotene solution (1 cc.) + 1 gram bile + 1 gram lipase	490	11	483	98.5 ±2.6
Carotene solution (1 cc.) + 1 gram bile + 1 gram lipase + 10 cc. H_2O (6 hours at 37°C.)	490	9	481	98.1 ±2.9
Immediate recovery of carotene from loop in presence of bile and lipase	490	12	466	95.3 ±3.4
Immediate recovery of carotene from loop in presence of bile and lipase	2450	7	2355	96.1 ±3.2

without a period of absorption. In these experiments, mixtures of known amounts of carotene in oil with bile and lipase were placed in the loops as described, and the loop contents were withdrawn immediately by suction on the rubber tube with a syringe. The loop was washed out three times with emulsions of 5 cc. of oil in distilled water. The loop contents and washings were combined and analysed in the usual manner. The averages shown in table 1, column five, lines five and six are satisfactory.

Effect of various substances on the absorption of carotene. In the absorption experiments a similar procedure was followed. Stock standard solutions of carotene in oil were placed in the loop along with the materials whose effects on the absorption were to be studied. Each solution was neutralized to pH 7 before introduction into the loop. After the absorption period, the loop contents were aspirated with a syringe, and the loop was

washed out several times with emulsions of oil and water as described above. The combined loop contents and washings were analysed and the

TABLE 2

Effect of various substances on the absorption of carotene from an intestinal loop

(Length of loop—8 inches; all absorption periods were 4 hours)

ABSORPTION CONDITIONS	NUMBER OF EXPERIMENTS	AMOUNT OF CAROTENE IN LOOP		CAROTENE ABSORBED (AVERAGE)	CAROTENE ABSORBED (PER CENT OF INITIAL)
		Initial	Final (average)		
		micrograms	*micrograms*	*micrograms*	
Carotene in 1 cc. of oil + 10 cc. of water	13	473	461	12	2.5 ±2.4
Carotene in 5 cc. of oil + 10 cc. of water	8	473	457	16	3.4 ±3.1
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 10 cc. of water	20	473	398	75	15.9 ±3.8
Carotene in 1 cc. of oil + 1 gram of dried pancreatic lipase + 10 cc. of water	19	473	428	45	9.5 ±3.6
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 1 gram of dried lipase + 10 cc. of water	23	473	262	211	44.6 ±6.3
Carotene in 1 cc. of oil + 1 gram of dried ox bile + 1 gram of dried lipase + 10 cc. of water	20	473	229	244	51.6 ±5.6
Carotene in 1 cc. of oil + 0.74 gram of sodium glycocholate* + 1 gram of dried lipase + 10 cc. of water	15	473	309	164	34.6 ±4.9
Carotene in 1 cc. of oil + 0.65 gram of sodium cholate + 1 gram of dried lipase + 10 cc. of water	15	473	294	179	37.8 ±4.1
Carotene in 1 cc. of oil + 0.63 gram of sodium desoxycholate† + 1 gram of dried lipase + 10 cc. of water	15	473	162	311	65.7 ±6.3
Carotene in 1 cc. of oil + 1.2 grams of dried hog bile + 1 gram of dried lipase + 5 cc. mineral oil + 10 cc. of water	5	473	425	48	10.1 ±5.5

* Synthesized by the method of Cortese and Bauman (7).

† Courtesy of Riedel-de Haen, Inc.

amount of carotene absorbed was determined by the difference between the amount of carotene placed in the loop initially and the amount remaining after the absorption period. The results are recorded in table 2.

All results presented in table 2 were obtained with the same loop dog in order that direct comparisons may be made between the different absorption conditions. However, similar experiments have been performed with three other dogs with comparable results.

Effect of carotene concentration on its absorption. In order to determine the effect of the initial concentration of carotene on the rate of absorption of this provitamin from the intestine, numerous absorption experiments were conducted for various absorption intervals with two different initial amounts of carotene (500 and 975 micrograms) in cotton-seed oil. In each experiment 1 gram of dried lipase[2] and 1 gram of desiccated ox gall-bladder bile[3] were added, and the mixture was washed into the loop with 10 cc. of distilled water. The results plotted in figure 2 were obtained with the same dog for comparison. All points shown in this graph represent the average of ten different experiments. The rate of absorption is somewhat greater when the larger initial concentrations of carotene are employed.

DISCUSSION. It may be seen from table 2 that the absorption of carotene from the loop in the presence of oil but in the absence of bile and lipase is very slight if, indeed, it is significant at all. When lipase is added, a small but statistically significant amount of carotene is absorbed. Similarly, significant absorption occurs in the presence of hog bile alone. However, when both one gram of dried hog bile and one gram of desiccated lipase are placed in the loop with the carotene solution, much larger amounts of carotene are absorbed during a period of the same duration. The effect of bile on the absorption of fat-soluble vitamins has received much attention, but the beneficial effect of pancreatic lipase on such absorption has not been emphasized. However, recently May et al. (18) have indicated that absorption of carotene is defective in children with pancreatic disease or obstruction of the pancreatic duct as well as in those with biliary obstruction. Our results would suggest that the defective absorption in such cases may be due to deficiency of pancreatic lipase. The decrease in the absorption of carotene in the absence of pancreatic lipase may be explained as due to disturbed fat digestion and absorption which would lead to retention of the fat-soluble carotene in the intestine. In this regard, the effect of mineral oil on the absorption of carotene may be commented upon. Table 2 indicates that mineral oil placed in the intestinal loops decreases the absorption of carotene even in the presence of bile and lipase. Such an effect has been reported by others (4, 9). This also may be explained on the basis of the solubility of carotene in this oil. Since only traces of mineral oil are absorbed, the oil-soluble carotene will be retained in the intestine.

Hog and ox gall-bladder bile are almost equally effective in promoting

[2] Difco Laboratories.

[3] Desiccated hog and ox gall-bladder bile were generously donated by Parke, Davis and Co.

absorption of carotene. In table 2 it would appear that ox bile is somewhat more effective than an equivalent quantity of hog bile, but this difference has little statistical significance. The amounts of desiccated ox and hog gall-bladder bile used in these experiments were equivalent (on a mol basis) in their content of total bile salts. The effectiveness of hog bile is interesting in view of the fact that the bile acids of hog bile are quite different qualitatively from those found in dog bile (19). On the other hand, the bile acids of dog bile and ox bile are quite similar, the only important difference being that ox bile contains bile acids conjugated with glycine as well as with taurine (19).

The results of experiments on the effect of pure bile salts on the absorption of carotene are interesting from the standpoint of the theories concerning the mechanism of absorption. In these experiments (recorded in table 2) the amounts of bile salts used were equivalent on a mol basis to the total bile salt content of the gall-bladder bile used in the previous experiments. It may be seen that the unconjugated bile salts are more effective than the conjugated salt. The positive results with sodium glycocholate are contradictory to the experiments of Greaves and Schmidt (15), but the conditions are not entirely comparable since they used rats and their criterion for absorption was the restoration of a normal vaginal smear picture. Of the unconjugated bile salts, sodium desoxycholate has greater effect than sodium cholate. These results may be interpreted in relation to the theory of Wieland and Sorge (23), Fürth and Minibeck (12) and Verzár (21, 22) which explains the effect of bile salts on the absorption of fat and fat-soluble materials as being due to the formation of choleate complexes with the bile salts. It has been pointed out (20) that stable, crystallizable choleate complexes are formed chiefly by desoxycholic acid and its salts. Von Euler and Klussmann (10) have described water soluble, diffusible complexes of the bile salts with carotene. Conjugation destroys the ability of desoxycholate to form stable, crystallizable choleates (8). However, in spite of their inability to form crystalline choleate complexes, conjugated bile salts such as sodium glycocholate are effective in promoting absorption of carotene (table 2). It should be pointed out also that bile salts in bile are principally in the conjugated form (19), but bile nevertheless is quite effective in aiding the absorption of carotene. Therefore, the importance of choleate formation in the rôle of absorption of this fat-soluble vitamin should be minimized although the concept cannot be abandoned completely since Verzár and Kuthy (21) have presented evidence that conjugated bile acids can form complex choleates in solution even though the complexes cannot be isolated and crystallized. The marked surface activity of bile salts which permits emulsification of fat and dispersion of the fat soluble vitamins probably plays an important rôle in the absorption of carotene, but this may not be the complete explanation of the mechanism.

SUMMARY

The absorption of carotene from isolated intestinal loops of dogs has been studied by means of photoelectric colorimetric determinations of the provitamin in the loop contents before and after absorption periods. Carotene is not absorbed in an analytically significant amount when placed in the loops in concentrated solutions in cotton-seed oil without bile and pancreatic lipase. When carotene solutions are given along with hog or ox gall-bladder bile, significant amounts are absorbed. Similarly, small amounts are absorbed when carotene in oil is introduced into the loops with pancreatic lipase. However, when the carotene solution is placed in the intestinal loops with *both* bile and pancreatic lipase, much larger amounts are absorbed.

Pure bile salts are capable of promoting the absorption of carotene. Sodium desoxycholate is the most effective bile salt of those studied, but sodium cholate and sodium glycocholate are also effective. These results and other evidence in the literature suggest that the choleate theory of the mechanism for the absorption of carotene should be minimized but not abandoned completely.

In the presence of identical amounts of bile and lipase, the rate of absorption of carotene is greater when the initial amount of carotene in the loop is increased. Reports on the inhibiting effect of mineral oil on the absorption of carotene are confirmed.

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THE EFFECT OF THIAMIN HYDROCHLORIDE ON THE MUSCULAR DYSTROPHY OF AVITAMINOSIS-E

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Several years ago Evans and Burr (1928) described a paralysis which developed in suckling rats that were fed a vitamin E deficient ration. They concluded that the paralysis was a true deficiency disease which could be produced or prevented by regulating the amount of vitamin E consumed by the young rats. Olcott and Mattill (1934) observed paralysis in 30 to 40 young mother rats which had been fed a vitamin E free diet for about four weeks. Ringsted (1935) reported paresis in adult rats that had received an inadequate supply of vitamin E for four to seven months. Blumberg (1935) also noted muscular disturbance and emaciation in female rats fed a vitamin E deficient ration for forty to fifty weeks. Morgulis and Spencer (1936) were able to prevent or cure muscular dystrophy in rabbits by supplementing the experimental ration with materials containing vitamin E but they concluded that paralysis and muscular dystrophy are not due to the same cause. Burr, Brown and Moseley (1937) state that vitamin E paralysis is easily induced in suckling young rats, that old age paralysis may also be due to vitamin E deficiency and that the paralysis is neither completely motor nor completely sensory. Morgulis, Wilder and Eppstein (1938) concluded that of the essential nutritional factors necessary for the integrity of the skeletal muscles the fat-soluble one is very closely associated or perhaps identical with vitamin E. This survey of the literature indicates that these and other investigators are in agreement that the various forms of muscular dystrophy occur in animals receiving vitamin E deficient rations.

Olcott (1938) commented upon the sudden occurrence of paralysis in second generation rats and stated that the paralysis in the young of vitamin E deficient female rats is due to lesions of the skeletal muscle similar to those in so-called nutritional muscular dystrophy of herbivora and he apparently considers this condition due largely if not wholly to the lack of vitamin E.

During the course of conducting vitamin E assays in this laboratory three female rats which were being maintained on ration I, a modified Olcott and Mattill (1934) ration, consisting of casein 20 per cent, sucrose

44 per cent, lard 22 per cent, yeast 8 per cent, salt mixture 4 per cent, and cod liver oil 2 per cent, produced litters of living young. At approximately weaning age the young rats developed various degrees of muscular dystrophy and paralysis. They were immediately weaned but continued to receive the same vitamin E deficient ration. When the young rats no longer received any of their mothers' milk and were entirely dependent upon ration I they began to improve. After a short time they appeared normal and grew to maturity. In seeking a possible explanation for this situation, attention was directed to vitamin B_1 and some experiments were conducted to determine the effect of administering thiamin hydrochloride to weanling rats afflicted with muscular dystrophy and paralysis.

Litter 4, of ten rats, was born to a female with avitaminosis-E. This animal had been fed ration II, a modification of Mason and Bryan (1938) diet 68. Its approximate composition was: casein (commercial) 20.0 per cent, corn starch 49.1 per cent, lard 18.2 per cent, Osborne and Mendel (1913) salt mixture 3.6 per cent, brewer's yeast 7.3 per cent and cod liver oil 1.8 per cent. Five of the young were raised and when they were twenty days old two died during the night. Previously they did not show any symptoms of muscular dystrophy, received no medication and the exact cause of their death was unknown. Two others developed pronounced but not severe muscular dystrophy. These animals were sufficiently active to be able to eat their vitamin E deficient ration in the usual manner. Two doses of thiamin hydrochloride, of six gamma each, were administered by mouth. Definite improvement was shortly evident and apparently complete recovery was obtained within a few days. The fifth young rat developed severe muscular dystrophy on the twentieth day of life. It refused all food and was fed, with a medicine dropper, the vitamin E-deficient ration II, mixed with distilled water. This was supplemented with a solution of vitamin B_1 (thiamin hydrochloride), six gamma, administered by mouth. Definite improvement and increased appetite were noted within twenty-four hours. However, it was still necessary to feed ration II mixed with water by a dropper and the rat still dragged its hind legs. The following day a second dose of twelve gamma of vitamin B_1 was administered. The rat made decided improvement and ate of its own accord. Two days later it ate and walked normally and was apparently cured without further treatment.

Litter 5 was born to a female which had received ration II continuously since she was fourteen days old. The five young rats developed pronounced muscular dystrophy at twenty-two days of age. A dose of six gamma of thiamin hydrochloride was administered to each animal during the morning. In the late afternoon each animal received an additional twelve gamma of thiamin hydrochloride. One rat developed an enlarged head (edematous) which also improved following the administration of

synthetic vitamin B_1. In two days these rats were cured with the exception of a slight flexure of the toes which later corrected itself without further dosage. In all instances the young rats were kept on the same vitamin E low diet as their mother except in the case where forced feeding was necessary for existence, then the diet was mixed with distilled water and fed by a medicine dropper.

Litter 6 was produced by a female that had been fed ration II from the fourteenth day of life. The entire litter of five rats was raised to twenty days of age. At that time two of the animals died suddenly during the night without having exhibited definite symptoms of muscular dystrophy. Previous to their death these rats apparently were definitely uncomfortable although they were very active. Three of the litter of five which were raised to weaning developed severe muscular dystrophy at twenty days of age. During the twentieth night one of the young rats died without having received any medication. The two remaining rats developed very severe muscular dystrophy. They became badly emaciated and refused to eat. A dose of fifteen gamma of vitamin B_1 thiamin hydrochloride, in solution, was administered by mouth. Due to difficulty in administration approximately only one-half of this amount was consumed. Little or no change in the animals' condition was noted except that there was a slight increase in an effort to take food from a dropper (vitamin E deficient ration II mixed with distilled water). Previous to the administration of vitamin B_1, the rats exhibited an extremely helpless and dazed condition (as in infancy). Two days later twelve gamma were again given by mouth to each rat. Late in the afternoon each animal was given a second dose of twelve gamma of thiamin hydrochloride. A decided improvement followed. The animals showed definite interest in food although refusing to eat of their own accord. The next day twelve gamma of thiamin hydrochloride were administered to each rat. There was a further slight improvement in physical condition but the appetite was decidedly improved. Five days from the onset the rats were apparently free of the muscular dystrophy except a slight flexure of the toes remained. The sixth day after medication was started the animals were free from the flexure of the toes and seemed to be completely cured. A total of approximately forty-three gamma or fourteen International Units of thiamin hydrochloride was administered to each animal during a period of four days.

The amount of thiamin hydrochloride which was required to produce recovery in the afflicted rats varied from twelve to forty-three gamma or from four to fourteen International Units of vitamin B_1. Apparently the more severe the condition of the rat, the larger the amount of thiamin hydrochloride required.

A survey of the literature did not reveal any instance in which this

amount of thiamin hydrochloride had been administered within four days to weanling rats of about forty grams weight suffering with muscular dystrophy following the continuous feeding of their mother with a vitamin E-deficient ration. However, the results obtained in this study indicate that the recoveries which followed the administration of vitamin B_1 to young rats with muscular dystrophy and paralysis resulted from the massive doses administered.

As further evidence that the administration of massive doses of thiamin hydrochloride caused a permanent cure of the weanling rats afflicted with muscular dystrophy and paralysis, all the rats grew to maturity and have remained in good health during six months of adult life.

SUMMARY

Studies upon young weanling rats which had developed various stages of muscular dystrophy, due to a vitamin E-deficient diet, revealed that there was a definite response to the oral administration of massive doses of vitamin B_1 (thiamin hydrochloride).

The response of the individual rat to a definite dose varied according to the severity of muscular dystrophy.

The total amount of thiamin hydrochloride necessary to effect a cure ranged from 4 to 14 International Units per rat.

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THE PENETRATION OF RADIOACTIVE SODIUM AND PHOSPHORUS INTO THE EXTRA- AND INTRA-CELLULAR PHASES OF TISSUES[1]

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Sodium has received considerably less attention in the study of tissue electrolytes than chloride, undoubtedly because of the difficulty of sodium micro-chemical methods. It has been generally assumed to be in the extracellular phase because of the evidence that most of the chloride in muscle is extracellular (7) and because of the fact that in some tissues the sodium:chloride ratios are the same as in plasma. The tendency to extend to all tissues, concepts which may aptly describe muscle, was criticized by Amberson et al. (1) and by Manery and Hastings (18), the latter showing that the sodium:chloride ratio differed appreciably in many tissues from that in plasma. An "excess" chloride was reported (18) in rabbits in blood, connective tissues, testes, gastric mucosa and in the liver of rats (13a, and this paper), and an "excess" sodium in cartilage, spinal cord and intestinal wall (18) which suggests the existence of both intracellular sodium and chloride. It is of some interest, too, that in certain muscles a small "excess" sodium has been reported but this is not true for all muscle tissue. It was located in frog (6) and in dog skeletal muscle (11, 12) but not in the skeletal muscles of the rabbit (11, 18). To conform to the simple morphological division into intra- and extracellular phases, this excess sodium has been allocated to the intracellular phase. In view of these findings and of the fact that the red cells of many species (15) contain sodium instead of potassium, it seems possible for sodium to function as an intracellular ion. This report shows how radioactive isotopes were used to investigate such a possibility.

The general scheme of dividing the tissues into two phases, intra- and extracellular, has been adopted. The limitations of this simple procedure are fully realized but its lack of complexity makes it useful. The following studies have been carried out and are reported herein:

a. The rate and extent of the penetration of radioactive sodium into a

[1] The data reported here were presented at the meeting of the American Physiological Society, Toronto 1939 (see (16)).

variety of mammalian tissues with a view to determining the proportion of sodium already present as intra- or extracellular; *b*, a comparison of the behaviour of radioactive phosphorus, phosphate being typically an intracellular ion, with that of sodium which is essentially extracellular; *c*, the penetration of both isotopes into red cells; *d*, the use of radioactive sodium for the determination of the extracellular fluid of the organism as a whole.

Methods and calculations. Sodium chloride and red phosphorus were bombarded[2] with deuterons to obtain radioactive sodium ($_{11}Na^{24}$) of half-life 14.8 hours, and radioactive phosphorus ($_{15}P^{32}$) of half-life 14.5 hours, respectively. Before the sodium chloride was used, sufficient time elapsed for the complete dissipation of the radioactivity due to the chlorine isotope which is produced simultaneously.

The sodium isotope was given to animals in 0.8 to 1.0 per cent solutions, 2.5 to 3.0 ml. per 100 grams of body weight being injected intraperitoneally into rats and $\frac{1}{10}$ of this dose into the ear veins of rabbits. The solutions contained not more than 2.0 millicuries of radioactivity per liter. The radioactive phosphorus was injected as a trace of sodium dihydrogen phosphate dissolved in an isotonic sodium chloride solution, the quantity of solution being the same as in the case of radioactive sodium. The animals were killed by decapitation at various periods of time after injection. They were bled and the tissues, plasma, peritoneal fluid and urine sampled and prepared for analysis. Tissues were freed from large blood vessels, connective tissue capsules and fascia where possible. In the kidney an attempt was made to exclude the medulla and use only cortex. Chemical determinations of chloride and sodium were made according to the procedures and with the accuracy previously described (17, 18).

The radioactivity of each sample was determined by means of a Geiger-Müller counter employing the counting tube and technique described by Bale et al. (2). Because this apparatus was designed to determine the activity in 2 ml. of solution, two methods of ashing the tissues were utilized. In some experiments they were dissolved in nitric acid on a steam bath. Weighing bottles were used and the solution diluted to the desired volume and weighed. Since a 2 ml. aliquot was taken for analysis the specific gravity of the solution had to be known. Although this is an expedient method it is more hazardous and hence less desirable than the second procedure adopted, namely, ashing in a muffle furnace overnight at about 530°C. The latter method is particularly convenient because the ash can be dissolved in 1 ml. of N HCl and diluted with water at the time of counting to any volume necessary to bring the number of counts per minute within the range for which the instrument has been calibrated.

[2] The radioactive isotopes were prepared in the Department of Physics through the courtesy and coöperation of Dr. L. A. DuBridge and Dr. S. N. Van Voorhis, and with financial support from the Rockefeller Foundation.

All solutions were counted for 3 to 5 periods of 5 minutes and each figure reported is the average of the counts for several periods. To calculate the results a distilled water background count was first subtracted from each unknown which was then calculated to an arbitrarily chosen but constant potassium acetate count. With an isotope of short half-life a standard radioactivity decay curve was employed to take account of the decay which occurred during the course of the experiment.

The accuracy obtained with this method may be expressed as the per cent difference between the counts given by duplicate tissue samples, which is 7 per cent (average of 20 pairs).

The counts alone for each tissue are quite meaningless, but their relative values are informative when related to some constant such as the number of counts injected or the plasma count. The plasma is a better point of reference than whole blood because of the possible accumulation of the isotope in the blood cells. We have calculated the ratio of the tissue concentration to the plasma concentration of *a*, radioactive sodium (Na^{24}); *b*, chemically determined sodium (Na), and *c*, chemically determined chlorine (Cl). In any tissues where all of the sodium and chloride are extracellular the sodium and chloride will be contained in the interstitial fluid which is undoubtedly a plasma ultrafiltrate. Hence when proper corrections have been applied to the above ratios for the water content of plasma and the Gibbs-Donnan equilibrium (see below), the resulting values will measure the volume of the extracellular water. In keeping with symbols previously used, this calculated value has been designated $(H_2O)_E$. Such a calculation obviates any assumption with regard to the solid content of the extracellular phase (see Manery and Hastings, 18). It is not our belief that sodium and chloride are entirely extracellular in all tissues of the body (5, 17, 18). However, the analytical figures for each tissue were subjected to the same method of calculation, for convenience in comparing the tissue: plasma ratios.

The method of calculation follows:

$$(H_2O)_E\,Cl = \frac{\text{tissue Cl (m.eq. per kgm.)}}{\text{plasma Cl (m.eq. per liter)}} \times 0.95 \times 0.93 \times 100 \quad (1)$$

$$(H_2O)_E\,Na = \frac{\text{tissue Na (m.eq. per kgm.)}}{\text{plasma Na (m.eq. per liter)}} \times \frac{0.93}{0.95} \times 100 \quad (2)$$

$$(H_2O)_E\,Na^{24} = \frac{\text{tissue counts per min. per kgm.}}{\text{plasma counts per min. per liter}} \times \frac{0.93}{0.95} \times 100 \quad (3)$$

$$(H_2O)_E\,P^{32} = \frac{\text{tissue counts per min. per kgm.}}{\text{plasma counts per min. per liter}} \times 0.95 \times 0.93 \times 100 \quad (4)$$

Where $(H_2O)_E$ is expressed in grams per 100 grams of fresh tissue, 0.95 is the Gibbs-Donnan ratio and 0.93 the correction for the water content of plasma. These values may be referred to in the text as Cl, Na, Na^{24} and P^{32} ratios respectively.

In addition calculations were made of the proportion of the body into which the absorbed radioactive sodium was distributed. If sodium is entirely extracellular this value will represent the volume of the extracellular water in the body as a whole.

Per cent distribution (gram per 100 grams body wt.) =

$$\frac{\text{Counts injected} - \text{counts excreted}}{\text{plasma counts per liter}} \times \frac{930}{0.95} \times \frac{100}{\text{body wt. (gram)}} \quad \ldots (5)$$

The amount of the dose injected which is found in the plasma at the time of decapitation is of some significance. Using Skelton's values (19) for the blood contents of rats and rabbits which are 5.5 and 6.75 per cent of the body weight respectively, the calculation is as follows:

Per cent dose in plasma =

$$\frac{\text{per cent pl.} \times \text{per cent bl.} \times \text{body wt. (gram)} \times \text{pl. count per ml.}}{\text{counts injected} \times 100} \quad \ldots (6)$$

Where per cent pl. = plasma volume in per cent of whole blood.
and per cent bl. = blood content in per cent of body weight.

RESULTS. 1. *Na^{24} distribution and the extracellular phase.* Because many tissues were analysed in each of the 18 animals studied it will be impossible to present all results in detail. The actual experimental figures are given in the case of only one animal (table 1) but sufficient data are presented in the other tables to permit calculation of any desired analytical values.

a. *Na^{24} distribution at equilibrium.* The first experiment (table 1) was designed to ascertain the extent to which tissues were penetrated by injected radioactive sodium, in a period of time adequate for equilibrium to be established. To decrease the sampling error both chemical sodium and radioactivity were determined on the same sample. In columns (1) and (2) are listed the chloride and sodium concentrations of tissues. These compare well with those previously determined (18) on normal rabbits receiving no injections, showing that neither the solution injected nor the radioactivity of the sample appreciably altered the normal electrolyte concentrations. The values for $(H_2O)_E$ in liver, brain, perirenal fat and all muscles are the same whether calculated from chloride or sodium. In tendon, kidney, testes, spleen, parts of the gastrointestinal tract and bladder the chloride ratios exceed the sodium values. In cartilage and sciatic nerve the reverse is true. These results, although uncorrected for blood and fat, in general confirm earlier findings. It has already been suggested

TABLE 1

Analyses and calculations pertaining to rabbits injected intravenously with radioactive sodium

FRESH TISSUE	RABBIT 1 (1 HR. 8 MIN.)							RABBIT 2 (13 MIN.)
	m.eq. per kgm. or l. of		Counts per min. per m.eq. ÷ 10^3	Per cent dose of Na^{24} in whole organ	$(H_2O)_E$ in grams per 100 grams calculated from			
	(1) Cl	(2) Na	(3) S.R.	(4)	(5) Cl	(6) Na	(7) Na^{24}	(8) Na^{24}
Skin	67.8			11.6	62.5			42
Tendon	80.9	74.6	13.0		74.6	52.1	53	39
Kidney*	55.7	65.6	11.8	1.2	51.3	45.8	42	33
Testes*	52.6	40.7	12.5		48.5	28.4	28	18
Ear cartilage	60.3	138.6	13.9		55.6	96.6	104	71
Spleen	46.6	43.2	11.8	0.1	43.0	30.1	27	23
Liver*	24.2	32.5	12.7	4.7	22.3	22.6	22	17
Pyloric mucosa	72.8	47.5	14.2		67.1	33.1	36	33
Pyloric muscle	47.0	36.9	14.3		43.3	25.7	29	23
Fundic mucosa	119.6	25.2	12.2		110.1	17.6	17	
Fundic muscle	72.6	42.0	12.1		67.0	29.3	27	
Small intestine	40.7	47.7	12.2	5.1	37.5	33.2	32	
Sciatic nerve	49.5	77.7†	6.6	0.2	45.7	*54.2*	*28*	
Cerebrum	39.9	51.5	3.8	0.3	36.8	*35.9*	*11*	4.3
Bladder	62.6	62.9	11.7		57.8	43.8	40	49
Heart	32.0	42.6	13.2	1.1	29.5	29.7	30	28
Abdominal muscle	23.6	25.4	13.9	31.0	21.8	17.7	19	11
Gastrocnemius	13.1	14.4	13.1		12.1	10.0	10	7.7
Perirenal fat*	7.9	11.0			7.3	7.7		
Urine*	13.1	Negl.						
Plasma*	95.8	140.1	12.9					
Whole blood*	85.4	98.7	12.0	17.6				
Rest of body				2.1				

Per cent distribution (grams per 100 grams body weight), for rabbit 1 = 25, for rabbit 2 = 21.

Per cent of injected dose in plasma, for rabbit 1 = 14, for rabbit 2 = 20.

Negl. = negligible.

Rabbit 1 was killed in 1 hour 8 minutes after intravenous injection, rabbit 2 in 13 minutes. Tissues of rabbit 1 were ashed in a muffle furnace for Na and Na^{24} determinations, those of rabbit 2 ashed in nitric acid on a steam bath.

Liter units apply to urine, plasma and whole blood; kilogram units to all other tissues.

* Figures pertaining to these tissues are averages of duplicates. All others represent single analyses.

† From Manery and Hastings (18).

(18) that the sodium figure gives a more reasonable value for the extracellular phase than the chloride figure in a tissue in which the chloride ratio exceeds the sodium ratio.

If the sodium is entirely extracellular it must be in ionic equilibrium with plasma and any injected radioactive sodium should freely exchange with sodium already present in the tissues. In this case the volume of the extracellular water based on Na^{24}, i.e., $(H_2O)_E Na^{24}$, should be equal to $(H_2O)_E Na$. If, on the other hand, some of the sodium is contained within cells which are impermeable to it, $(H_2O)_E Na$ will exceed $(H_2O)_E Na^{24}$ by the amount of non-diffusible intracellular sodium present. By comparing the values of extracellular water in column (6) with the corresponding figure in column (7) a striking agreement is demonstrated in every tissue studied except brain and sciatic nerve. The ratios are almost identical in many tissues and even the greatest difference does not exceed 10 per cent, whereas in brain and nerve the Na^{24} ratio only attains 30 and 50 per cent respectively, of the magnitude of the Na ratio. There is no correspondence whatsoever between the quantity of extracellular water calculated from Cl and that from Na^{24}, even in nerve and cartilage where the sodium concentration exceeds the chloride. Furthermore, there is no evidence of a tendency for the Na^{24} ratio to exceed that of chemical sodium showing that the radioactive isotope does not concentrate in any tissue. The experiment demonstrates that, with the exception of nervous tissue, in 1 hr. 8 min. injected radioactive sodium becomes distributed between tissues and plasma in precisely the same ratio as normally occurring sodium.

In column (3) we have listed calculations of the counts per milliequivalent of sodium $\div 10^3$ (S.R. = specific radioactivity) found in each tissue. If tissue sodium is in free diffusion equilibrium with plasma sodium then Na^{24} should mix with the tissue sodium in the same proportion as it does with the plasma sodium. The value of S.R for plasma is 12.9. The table shows that for all tissues except nervous tissues S.R. very closely approximates the plasma value. Actually, excluding cerebrum and nerve, the average is 12.8. The close correspondence of tissue S.R. values to the plasma value merely illustrates in another way the completeness with which Na^{24} has become mixed in an hour with the sodium of the body.

Bladder urine contained very little chloride and insufficient sodium to be analyzed by this chemical method. The radioactivity was also negligibly small at the time at which other tissues were counted. Hence the data obtained on this animal are so nearly complete that we have attempted to account for the dose injected by estimating the amount found in each organ. Skelton's tabulation (19) of the percentage weight of rabbit organs in relation to body weight was used and also the Na^{24} concentration found for skin in rabbit 3. The results are listed in column (4) in per cent of the dose injected. Muscle, whole blood and skin contain much the largest proportion of the injected sodium. The sum for all organs is 75 per cent but we have omitted the skeleton which makes up 12.4 per cent of the body weight. By assigning 25 per cent of the counts injected to the

skeleton and assuming the value of S.R to be that of plasma the concentration of sodium was calculated to be 71 m.eq. per kgm. of skeleton. This concentration, no doubt fortuitously, is exactly that reported by Harrison et al. (11).

b. *Rate of penetration into tissues.* Since radioactive sodium in one hour leaves the plasma and becomes distributed throughout tissues, in the manner already described, the first interpretation suggested is that all tissue sodium in the body is extracellular and in free diffusion with plasma sodium. On this basis nervous tissue exhibits a certain degree of impermeability. That it is not complete but merely a relative impermeability causing delayed entrance is to be expected, and is clearly demonstrated by allowing longer periods of time for penetration than one hour (tables 1 and 2). Since the completion of this work Hahn, Hevesy and Rebbe (9) have published analyses on rabbit tissues 67 hours after injection. The value of $(H_2O)_E Na^{24}$ calculated from their data is 31 grams, which although chemical sodium was not determined cannot differ greatly from $(H_2O)_E Na$.

This relative impermeability of brain to injected sodium suggested that if tissues had intracellular sodium phases, differential rates of entrance into them might exist. Whereas 3 to 12 hours were required for Na^{24} to enter brain it might enter the sodium-containing phase of other tissues much more rapidly. In fact it should diffuse almost instantaneously into the extracellular phase and, although delayed in entering cells, this too might be complete in an hour. Experiments were designed to determine the rate of entrance into tissues in shorter periods of time than 1 hour. Penetration was taken to be complete when the Na^{24} ratio equalled the Na ratio, and as an initial premise a delay in attaining completeness was considered indicative of an intracellular sodium fraction.

Rabbit 2 was decapitated in 13 minutes after injection and the results are given in column (8) table 1. The situation is somewhat complicated in this animal by the fact that complete equilibrium was not yet attained. Plasma samples were analysed at 3 minute intervals during this period to follow the rate of disappearance of radioactivity. Although the curve showed a tendency to flatten out in 13 minutes (see Griffiths and Maegraith, (8) who report constancy in 15 to 20 minutes), nevertheless 20 per cent of the dose injected was still in the plasma and the per cent distribution was appreciably lower than in rabbit 1. The penetration seemed to be less complete in certain tissues of rabbit 2 than of rabbit 1, notably in testes and brain.

In order to obviate individual differences, the next animal (rabbit 3) was anesthetized and the same tissue sampled over a period of 3 hours. The operative procedure was considered too drastic to keep the animal in the best physiological condition, but nevertheless some of the results are worth recording. There were indications that more Na^{24} had entered

skin, tendon, kidney, liver and muscle in $1\frac{1}{2}$ hours than in 17 minutes. The following figures serve to confirm and extend those in table 1: In liver in 1 hour the Na^{24} and Na ratios were 18 and 19 respectively; in perirenal fat 14 and 12; in 3 hours the Na^{24} ratio in cartilage was 93, in gastrocnemius 12, in cerebrum 29 and in ovaries 29. From this one result Na^{24} seems to be delayed in its entrance to ovaries because the Na ratio calculated from the chemical sodium analyses of Manery and Hastings (18) is 35.

This rabbit was pregnant and because relatively few studies of electrolytes in the *reproductive organs* have been carried out some of these tissues were analysed for radioactivity, sodium and potassium. In 3 hours after injection the values of the so-called extracellular water based on Na and on Na^{24} were 55 and 52 grams respectively in uterine muscle, 60 and 22 grams in the endometrium, and 52 and 9 grams in the embryos. As we have stated the animal was in rather poor condition but in this particular case Na^{24} penetrated uterine muscle but did not pass through the endometrium into the embryos. These tissues differ from most in containing rather high concentrations of both potassium and sodium. The sodium concentrations in uterus muscle, endometrium and embryos were 84, 93 and 79 m.eq. per kgm. of fresh tissue and the potassium concentrations were found to be 72, 50 and 60 m.eq. per kgm. in the corresponding 3 tissues.

Rats injected intraperitoneally were next used. In animals intravenously injected, the plasma count is falling with time until equilibrium is reached, whereas in the intraperitoneal injections the per cent of the dose injected remains relatively constant in the plasma (see table 2), Na^{24} being absorbed from the peritoneal cavity at about the same rate as it leaves the circulatory system to enter the tissues. However, contamination of abdominal tissues with the intraperitoneally injected fluid can give rise to considerable error in short time experiments. Care was taken to minimize this source of error by removing the capsule of the kidney, sampling the liver from the interior, etc.

The results are summarized in table 2. In rats, too, we find the extracellular water based on Cl to be somewhat larger than that based on Na in some tissues, notably testes, stomach wall and liver whereas the sodium value seems to be slightly larger in abdominal, gastrocnemius and heart muscles. The analytical sodium and chloride concentrations in each tissue may be calculated by substituting the plasma concentration and the value of $(H_2O)_E$ Cl and $(H_2O)_E$ Na in equations (1) and (2).

The most complete data were obtained in rats 10 and 11, which show that in 3 hours excellent agreement was obtained between the Na and Na^{24} ratios in all tissues except testes and brain where the difference is 20 per cent, while in 12 hours the difference in testes seems to have disappeared

while that in brain persisted. In spite of the omissions the tissues can be divided into at least 2 groups; *a*, those in which the penetration of Na^{24} is complete in 20 minutes and remains constant for at least 12 hours, and *b*, those into which the penetration is delayed but gradually proceeds to completion in 3 to 12 hours.

TABLE 2

Values of $(H_2O)_E$ for tissues of rats killed at varying times after intraperitoneal injections of Na^{24}

TISSUE	RAT 1		RAT 2		RAT 3	RAT 4	RAT 5	RAT 6	RAT 7		RAT 8		RAT 9	RAT 10		RAT 11
	Rat weight (grams)															
	359		294		189	180	190	155					153	245		205
	3 min.		8 min.		8 min.	16 min.	20 min.	1 hr.	1 hr.		2 hr.		2 hr.	3 hr.		12 hr.
	Values of $(H_2O)_E$ (grams per 100 grams fresh tissue) calculated from															
	Cl	Na^{24}	Na	Na^{24}	Na^{24}	Na^{24}	Na^{24}	Na^{24}	Cl	Na^{24}	Cl	Na^{24}	Na^{24}	Na	Na^{24}	Na^{24}
Skin	44.1	27	40.9	19	34						40.9	39		39.2	41	58
Abdominal muscle	14.0		18.5								16.2	22		18.1	19	
Kidney	40.4		37.3			46*	43*	41*	55.5		48.6	49	39*	38.8	41	44
Liver	20.7		18.3			20*	19*	19*	24.0	19	25.4	18	19*	17.7	19	20
Testes	56.3		30.6	22	20	14*	15*	21*	57.2	20			22*	30.7	25	29
Gastrocnemius	10.5	7.1	13.4	8.7	11	13*	11*	15*	12.3	14	12.1	15	15*	14.3	13	15
Gastro-intestinal tract	53.4		31.8						32.4†	29				34.4	31	29
Heart	22.2		35.7	33	12									31.6	31	27
Femur	20.8	7.2			33											66
Brain		3.1	30.2	5.5	1.9									31.5	25	24
Plasma Cl (m.eq. per l.)	113.2															
Plasma Na (m.eq. per l.)			142.0													
$\frac{Na^{24}\text{ perit. fluid}}{Na^{24}\ (H_2O)_E}$	14.3		4.7		3.0	2.0	1.6		1.1		1.1			0.86		
Per cent distribution‡			29		29	27	28		28		30			32		
Per cent dose in plasma	4.2		6.9		9.2	8.2	8.1	8.0	8.9		8.3		5.5	8.0		6.8

Tissues of rats 2 and 10 were ashed in muffle furnace, those of other animals in nitric acid on a steam bath.

* Averages of duplicate analyses.

† Tissue analysed is the wall of the small intestine. Other figures in this row represent analyses of stomach wall.

‡ For the calculation of these values it was assumed that no Na^{24} was excreted.

We are indebted to Dr. W. O. Fenn for experiments on animals 7 and 8.

Skin, kidney, liver and gastrocnemius muscle unquestionably belong to group *a* and, while the data are rather incomplete, it is probable that abdominal muscle, heart and the gastro-intestinal tract should likewise be included. There is no evidence of an accumulation of Na^{24} in heart

such as that reported by Griffiths and Maegraith (8). In group *b* are found testes, brain, and probably femur.

The values of the Na^{24} ratios for *liver* are remarkably consistent and always equal to the sodium ratio but lower than the Cl ratio. There is some evidence that liver cord cells do not contain chloride (20) but it seems reasonable to suppose that Kupfer cells and others of connective tissue origin may have an intracellular chloride fraction without a corresponding sodium complement (see 17). In this tissue, then, the calculation of extracellular water based on sodium is probably a more accurate representation of its true volume than that based on chloride.

The intracellular sodium fraction, which has been postulated to exist in mammalian *muscles*, is so small that it is hardly detectable by the radioactivity method as used here. This method will not detect differences of less than $\pm$ 7 per cent. However, the values of extracellular water based on Na^{24} in gastrocnemius muscles are somewhat less in rats 1 to 5 than in rats 6 to 11. There is some indication that only the chloride space was entered in 20 minutes and that more time was required to penetrate the entire sodium space. A careful investigation is necessary in which chloride, sodium and Na^{24} are more accurately determined on the same muscle. One conclusion is clear-cut, namely, that if there is any intracellular sodium in muscle it comes into equilibrium with plasma sodium in at least one hour. In this connection the experiments of Heppel (13) are pertinent. He injected radioactive sodium into animals raised on a potassium deficient diet and found that it quickly penetrated the extracellular phase of the muscle as measured by chloride, and then slowly entered the muscle fibres for 1 hour at which time it had penetrated the volume occupied by chemically determined sodium. The most reasonable explanation at the moment of these findings is that sodium has penetrated muscle fibre membranes hitherto believed impermeable to it.

Kidney, which likewise belongs to group *a*, deserves special mention because, although Na^{24} penetrates the entire sodium-containing portion very rapidly, it is likely that the tissue contains both intracellular sodium and intracellular chloride (5). The active reabsorption thought to occur in the distal tubules would be difficult to explain if it were otherwise. The rapid rate of entrance may result from the high speed of turnover of threshold substances which must indeed occur in view of rapid blood flow through the kidney.

Brain, peripheral nerve, testes and femur constitute group *b*, where there is unmistakably a delayed entrance of radioactive sodium into the sodium space. The question is raised as to whether this delayed entrance is a true manifestation of intracellular sodium.

Brain has a relatively high sodium content. If it were all extracellular the calculated extracellular fluid would be 30 to 35 per cent. But prac-

tically no Na^{24} had entered this area in 8 minutes and in both rats and rabbits the amount penetrating had increased but penetration was not complete even in 3 hours. Other demonstrations of the existence of a relatively impermeable barrier between plasma and brain tissue have been reported. Wallace and Brodie (21, 22) demonstrated that the administered anions, iodide, thiocyanate and bromide were distributed from the plasma into tissues in the same fashion as chloride in every tissue studied except brain. They found by direct analyses (23) that these anions had not entered the cerebrospinal fluid. Furthermore the ratio of chloride to administered anion for the tissue of the central nervous system was always the same as that in the cerebrospinal fluid while the ratio in liver and lung corresponded to that in the plasma. This was confirmed for bromide by Weir and Hastings (24). The hypothesis presented by both groups of authors that the cerebrospinal fluid represented the extracellular fluid of nervous tissue seems plausible.

The cerebrospinal fluid was not analysed for radioactive sodium in our experiments, but in the light of the work just discussed it may have been retarded in entering this fluid. Its behaviour simulates that of bromine and iodine (23), being greatly delayed but gradually entering the tissue in the course of several hours. We conclude that sodium injected into the circulatory system does not diffuse freely into the sodium phase of the brain.

Very little is known about electrolytes in the reproductive organs. *Testis* is like gastric mucosa in showing a chloride concentration in excess of sodium (see also 18). The sodium gives a more reasonable value for the extracellular fluid than the chloride, but Na^{24} enters only two-thirds of the total sodium space in 20 minutes and seems to be delayed in its entrance into the other third. There are two main types of cells in the testis, connective tissue of which the interstitial cells may be secretory, and cells of the seminiferous epithelium in the tubules which is composed largely of sex cells. The tissue is glandular and our present information will only permit conjecture. Possibly the delayed entrance is due to secretion which might be much slower than in the kidney, for example, or perhaps circulatory differences are partially responsible.

The data on *bone*, although somewhat sparse, are of interest because they illustrate another case in which delayed entrance of Na^{24} does not necessarily indicate intracellular sodium. The value of $(H_2O)_E$ Na calculated from the data of Harrison et al. (11) is 50 grams. Hence Na^{24} has not entered the entire sodium containing area in 8 minutes, although it may have done so in less than 12 hours in view of the completion of the penetration into cartilage in 1 hour (table 1). Harrison (10) found all the chloride of bone with its sodium complement in the organic phase. The excess sodium was associated with the inorganic part of the interstitial substance. The

rate of penetration of Na^{24} in this case may indicate the rate at which sodium is renewed in the inorganic compound of which it is a part.

c. *Discussion* of 1. In order to give these data some semblance of orderliness we have commenced with the initial premise that slow entrance of radioactive sodium into tissues is a manifestation of intracellular sodium. It was obvious at the outset that such a simple postulate would be quite inadequate. Radioactive sodium will certainly pass almost instantaneously from the plasma into areas which are in free diffusion equilibrium with plasma. Taken in conjunction with other evidence the rapid entrance into liver and muscle undoubtedly indicates the extracellular position of most of the sodium in these tissues. But it is not necessarily true that all areas into which penetration is rapid are extracellular. The kidney is a case in point providing the interpretation suggested above is tenable. Similarly, slow penetration may not always demonstrate intracellular sodium. This seems to be the case with brain and bone.

As more and more data accumulate it becomes increasingly evident that generalizations which embrace all tissues cannot be made with regard to electrolytes. Every tissue is a composite of many types of cells, each with a specific function and on this function the electrolyte distribution ultimately depends.

We may conclude from these data 1, that radioactive sodium will penetrate the extracellular phase of tissues very rapidly; 2, that in the case of secretory cells it may quickly enter an intracellular phase; 3, that a delayed entrance may demonstrate the presence of an intracellular phase, the entrance into which is controlled by intracellular processes, or it may illustrate the interposition of a barrier between the blood and the extracellular phase of certain tissues, e.g., brain.

2. *Na^{24} versus P^{32}*. Another method of elucidating the position of sodium in the animal organism is by comparing its behaviour to that of phosphorus which is known to be essentially an intracellular ion. Although several investigations have been published on the distribution of radioactive phosphorus in tissues (see (14) for review), none present suitable data for our purpose because, in general, the whole blood and not the plasma has been analysed for radioactivity. We have calculated (see table 3) the tissue:plasma ratio for P^{32} according to equation 4, and called it $(H_2O)_E\ P^{32}$, though obviously the symbol can have no real meaning when applied to an intracellular ion.

Radioactive phosphorus differs from radioactive sodium in regard to its distribution (tables 2 and 3). In the case of Na^{24} about 8 per cent of the dose administered intraperitoneally is found in the plasma in 8 minutes and this remains constant over several hours while the amount of P^{32} in plasma gradually decreases with time. P^{32} is probably subjected to meta-

bolic influences which produce a continual drain on plasma phosphorus. Plasma sodium, on the other hand, comes into equilibrium with a more or less finite volume which does not vary with time, and which therefore can most reasonably be identified with the morphological extracellular phase. Similarly the percentage of the body weight into which the administered dose becomes distributed, i.e., per cent distribution, is constant at about 30 per cent for Na^{24} but increases with time in the case of P^{32} exceeding 100 per cent in 20 minutes. Again the participation of phosphorus in intracellular processes is demonstrated.

TABLE 3

Calculations pertaining to rats injected intraperitoneally with radioactive phosphorus

TISSUE	RAT 12		RAT 13	RAT 14		RAT 15	
	7 min.		14 min.	20 min.		2 hrs.	
	Values of $(H_2O)_E$ (grams per 100 grams fresh tissue) calculated from						
	Cl	P^{32}	P^{32}	Cl	P^{32}	Cl	P^{32}
Skin	51.4	33	29	45.2	38	51.5	98
Kidney	41.8		104	48.5	242	45.3	787
Liver	23.5		90	30.3	193	26.1	1,007
Testes	51.4	58	56	55.0	30	52.9	50
Gastrocnemius	12.2	12	10	14.6	12	14.0	73
Stomach	58.8		89	53.3	124	51.5	388
Heart	24.7		48	28.6	65	23.6	265
Femur	24.9	36	61				
Brain	29.3	Negl.	Negl.	30.0	Negl.	30.9	Negl.
$\frac{P^{32} \text{ peritoneal fluid}}{P^{32}\ (H_2O)_E}$	28.9		23.6	13.9		3.8	
Per cent distribution			58.6	118		377	
Per cent dose in plasma	2.3		3.0	1.5		0.6	

Negl. = negligible.

Examination of individual tissues shows a fundamental difference between Na^{24} and P^{32}. In general, contrary to the behaviour of Na^{24}, the concentration of P^{32} in tissues rises with time, the value of $(H_2O)_E$ P^{32} approaching or exceeding 100 per cent.

As in the case with sodium there is a differential rate of entrance of P^{32} into tissues. The volume entered exceeds the $(H_2O)_E$ Cl in 14 minutes in heart, liver, kidney, stomach wall and femur and gradually increases to varying amounts in 2 hours. The gastrocnemius muscle is of some interest because the P^{32} ratio in 20 minutes is almost exactly equal to the chloride ratio, while this is far exceeded in 2 hours. Surely this is a manifestation of the penetration of inorganic phosphate into muscle fibres. The entrance

is less rapid into skin, testes and brain. In testes in 2 hours only a volume equal to $(H_2O)_E$ Cl has been entered. In the case of brain one would offer the suggestion that P^{32} is also retarded from entering the cerebrospinal fluid and tissue spaces at the hemato-encephalic barrier.

An attempt was made to observe the entrance both in vivo and in vitro of Na^{24} into the *red cells* of 3 rabbits and 5 of the rats studied. The sodium concentration was found to be low in the erythrocytes of these animals, being 20 m.eq. per liter of cells in the case of the rabbits and 17 in rats, values which correspond satisfactorily with those of 16.0 and 12 m.eq. per kgm. of rabbit and rat corpuscles respectively, found by Kerr (15). In both cases the figures are calculated from analyses of plasma, whole blood and hematocrit values. The cell sodium per liter of cells amounts to only about 14 per cent of the plasma concentration which, because of the error in hematocrit measurements and radioactivity estimates, is close to the limits of our determinations. To avoid using hematocrit figures S.R. (count per m.eq. $\div 10^3$) values were calculated for whole blood and plasma, and the ratio of Na^{23} in whole blood:Na^{23} in plasma compared to the ratio of Na^{24} in whole blood:Na^{24} in plasma. Instead of the two values of S.R. being equal and the ratios being the same as would be the case if complete exchange of Na^{24} with the intracellular sodium had occurred the differences ranged from 0 to 8 per cent with no consistent change with time. Hence the experiments were inconclusive with regard to the exchange of Na^{24} with the small amount of intracellular sodium present in these cells.

Dog erythrocytes, which were found to contain 107 m.eq. per liter of cells, were then studied in *in vitro* experiments. Five milliliters of whole blood were mixed with 1 ml. of strongly radioactive isotonic saline. Whole blood and plasma were analysed for radioactivity and sodium, and the unwashed cells for radioactivity. In 18, 36 and 64 minutes the cell counts were represented by 126, 175 and 219 respectively showing a gradual penetration of Na^{24}. In 18 minutes the value of S.R. was 2.3 for plasma and 1.7 for whole blood. If complete exchange of Na^{24} for the intracellular sodium had occurred these values would be equal. The ratio of Na^{23} in whole blood:Na^{23} in plasma was 0.9 while a similar ratio for Na^{24} was 0.7. This difference may indicate that all but 20 per cent of the cell sodium exchanged with Na^{24}. The work was not continued because of the appearance of a paper by Cohn and Cohn (3). However these authors washed dog erythrocytes with isotonic sodium chloride after exposure to radioactive solutions and did not include chemical sodium analyses. Using our value of 107 m.eq. per liter of cells the ratio of Na^{23} in cells:Na^{23} in plasma $\times 100 = 76$ whereas their corresponding ratios for Na^{24} did not exceed 65, indicating again that about 15 per cent of the red cell sodium had not exchanged with plasma sodium.

Again the comparison of the penetration of Na^{24} with that of P^{32} is of interest. A typical *in vitro* experiment with P^{32} using rabbit cells, carried out in the manner described above, shows the plasma count to decrease with time while the count of unwashed cells increased in the following manner: in 22 minutes 984, in 1 hour 2120, and in $3\frac{3}{4}$ hours 6500. Cells which were washed after $3\frac{3}{4}$ hours' exposure to radioactivity counted 4770. As in the case of Na^{24} the gradual penetration of radioactive phosphorus into red cells is illustrated.

3. *The use of Na^{24} to measure extracellular fluid.* The volume into which the absorbed radioactive sodium is distributed (in per cent of the body weight as calculated by equation 5) gives a value of 25 in rabbit 1 (table 1) and 29 in rats 2–8 (table 2). These are the same order of magnitude as those reported by Harrison et al. (11) who based their calculation on the assumption that all of the body chloride is extracellular, and are similar to the results obtained with sulfocyanate injections by Crandall and Anderson (4).

The term extracellular fluid should refer to the fluid in the interstitial spaces bathing tissue cells. But most of the methods used depend on the distribution of substances which enter the Cl-containing phase of tissues, and hence are in error by the amount of intracellular chloride present. Although injected Na^{24} eventually enters the entire Na phase of all tissues, the rapidity with which it is distributed throughout 29 per cent of body weight without further change in 2 hours strongly indicates that it diffused immediately into the true extracellular space. The following advantages seem to favour the Na^{24} method; 1, it can be quickly and accurately determined in very small amounts and in coloured solutions and would be particularly suited to simultaneous measurements of plasma and extracellular fluid volumes; 2, excretion will be slight in the short time required to enter the extracellular fluid; 3, Na^{24} will not enter erythrocytes in those animals whose red cells are high in potassium and it can easily be determined when the cells contain sodium instead of potassium; 4, most important of all, Na^{24} can be administered in a few milliliters of isotonic saline and not only is this solution non-toxic but it obviates the need of introducing any substance in abnormal amounts.

SUMMARY AND CONCLUSIONS

1. The distribution of administered radioactive sodium (Na^{24}) throughout mammalian tissues was compared to that of normally occurring sodium (Na) and chloride (Cl). The method depended on the comparison of the ratios of the tissue:plasma concentration for each substance.

If sufficient time elapsed (up to 12 hrs.) the Na^{24} distribution was identical to the Na distribution, showing that there was no complete

impermeability to injected sodium in tissue phases which already contained sodium.

2. Tissues differed in the rates at which Na^{24} entered the Na phase. In general, it penetrated the extracellular phases rapidly and the Na-containing intracellular phases more slowly, penetration of the latter being clearly demonstrated in dog erythrocytes. Entrance proceeded rapidly into the Na phase of skin, kidney, liver and muscles but was delayed in testes, femur and brain. The significance of these findings with regard to the intra- or extracellular sodium of specific tissues has been discussed.

3. Na^{24} slowly entered dog erythrocytes which contain sodium but could not be conclusively demonstrated to penetrate rabbit or rat red cells which have a low sodium concentration.

4. Many of the differences found between the distribution of Na^{24} and P^{32} illustrate the fact that sodium is essentially an extracellular ion while phosphorus is concentrated within cells. However the slow penetration of both into nervous tissues indicates that certain barriers may be imposed between the blood and the extracellular phase of tissues, and that the position of a substance as intra- or extracellular is not the sole determining factor.

5. The extracellular fluid as measured by radioactive sodium was 29 per cent of the body weight in rats.

6. Some sodium, potassium and chloride analyses of reproductive organs and embryos are included.

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THE ELECTRICAL RESPONSE OF THE KITTEN AND ADULT CAT BRAIN TO CEREBRAL ANEMIA AND ANALEPTICS[1]

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The present report is concerned with a comparative study of the kitten and the adult cat brain to cerebral anemia under varying conditions which include 1, the effect of analeptics on cerebral electrical potentials; 2, the sensitivity of spontaneous cortical activity to cerebral anemia as compared with that of the evoked acoustic cortical response, and 3, the age of appearance of both spontaneous and evoked potentials.

METHOD. Silver-silver chloride wick electrodes (1) were used, a monopolar lead on the cerebral cortex and the other indifferent one on the frontal bone, feeding a push-pull amplifier and inkwriter (Grass). The cortex was exposed under ether, the animal curarized and placed under artificial respiration and the ether was blown off before electrical activity was recorded. In some experiments a 10 per cent solution of metrazol was injected intravenously in doses varying from 0.05 to 0.6 cc. In other observations a saturated solution of strychnine was painted on the cortex. To determine the effects of sudden brain anemia on the persistence of electrical activity, the heart was quickly excised from the previously opened thorax.

RESULTS. *Normal spontaneous activity and age.* In the 2 to 6 day old kittens, electrical activity of the cerebral cortex is practically absent or of very low amplitude (5–20 microvolts). When present this activity consists of random waves. Nevertheless in these kittens electrical responses from the cortex in the region of the ectosylvian sulci can be evoked by auditory stimuli, namely, a sharp noise. Nine day old kittens exhibited random slow waves from the occipital and frontal regions, but considerable activity or irregular frequencies (amplitude about 50 microvolts) from the regions about the anterior and posterior ectosylvian sulci. From these areas, as in the younger kittens, electrical responses to auditory stimuli can be readily elicited. Only part of the spontaneous activity of this region may be due to background noise; for example, the spontaneous

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waves disappear approximately 15 seconds after complete cerebral anemia while responses to acoustic stimuli can still be evoked at this time and these responses are of the same or larger amplitude than those before cerebral anemia. Kittens, 19 to 25 days of age, exhibit considerable electrical activity in the same region with an amplitude varying from 20 to 100 microvolts, and a frequency from 1 to 35 per second (fig. B1). Though the faster components are prevalent, they are more irregular and slower, 21 per second, than those appearing in the adult, 40 per second. Compare figure 1, A1, B1 and C1, normals for a 3 day and a 25 day old kitten and an adult cat respectively.

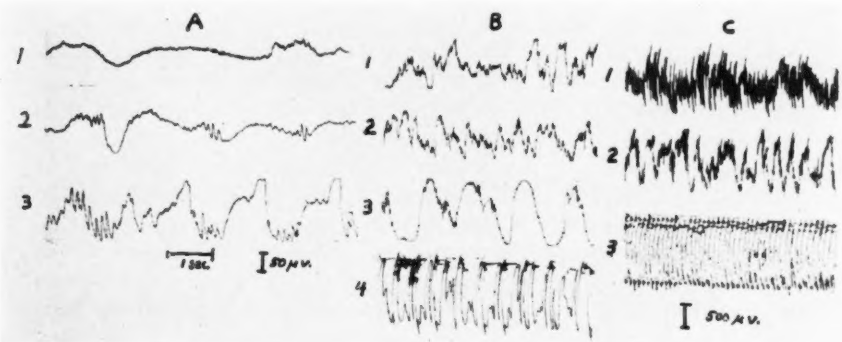

Fig. 1. Normal and metrazol activity of cerebral cortex in kitten and adult cat.

A. Three day old kitten. *1.* Spontaneous activity. *2.* After 0.1 cc. of 10 per cent metrazol intravenously. *3.* After another 0.1 cc. metrazol.

B. Twenty-five day old kitten. *1.* Spontaneous activity. *2.* After 0.1 cc. metrazol intravenously. *3.* After another 0.17 cc. metrazol. *4.* Another 25 day old kitten, 0.4 cc. metrazol into heart (had received 0.13 cc. metrazol into heart previously, which produced slow waves, resembling those in B2 here).

C. Adult cat. *1.* Spontaneous activity. *2.* Response after 0.2 cc. metrazol intravenously. *3.* Another adult cat—0.5 cc. metrazol intravenously.

All same amplification as shown below A3 (except C3—see below it).

Effect of metrazol and strychnine. The response to metrazol varies with the dose and the age of the animal. In the relatively silent cortex of 2 to 6 day old kittens electrical activity can be initiated by the injection of metrazol or strychnine, or the local application of the matter. In these newborn animals the intravenous injection of 0.05 cc. of metrazol usually resulted in sporadic bursts of activity for approximately 100 seconds. These often consisted of rather regular waves from 9 to 12 per second (20–50 microvolts) interspersed with spikes and slower, often diphasic components (fig. 1, A2). Larger doses of metrazol between

0.1 and 0.2 cc. initiated a more continuous activity of somewhat greater amplitude (fig. 1, A3). In many instances trains of large polyphasic waves appeared. The results with strychnine were similar to those observed with metrazol. In the 19 to 25 day old kitten, the injection of 0.1 cc. of metrazol accentuated the slow waves (fig. 1, B2). These were enhanced by a larger dose of 0.17 cc. (fig. 1, B3). Another kitten (fig. 1, B4) receiving a convulsive dose of 0.4 cc. of metrazol produced a train of spike-like waves similar to the adult but of lower frequency. Compare figure 1, B4 and C3.

The amounts of metrazol required to produce the central electrical responses or the peripheral motor convulsions were approximately the same, 0.15 cc. in 2 to 5 day old kittens. This is also true in the adult dog (2) and in humans (3). Like the electrical responses, the motor convulsions in the 4 day old kitten appeared to be different in nature from those of the adult. Instead of the violent clonic convulsions observed in the adult with metrazol, the kitten exhibits slower and smoother generalized movements of the limbs and trunk.

Cerebral anemia. The persistence of cortical electrical activity after rapid extirpation of the heart (an operation requiring less than 3 seconds) could be studied in the 2 to 6 day old kittens only after some electrical activity had been initiated by metrazol or strychnine. The survival time of such activity averaged 22 seconds. Though most activity disappeared by this time, in all cases occasional subsequent bursts of activity were noted from 39 to 100 seconds. After cardiac excision, the adult survival period, without the injection of metrazol, varies from 10 to 15 seconds. It does not appear probable that the longer survival time found in the 4 day old kitten, as compared with the adult, is due to use of metrazol. Older animals do not survive longer after receiving metrazol.

Evoked cortical response to acoustic stimuli. Bremer and Dow (4) have described the electrical response of the auditory area of the cat to acoustic stimuli. We have also observed responses to auditory stimuli in kittens (3-24 days old, as already mentioned above) and adult cats, with the active monopolar lead on the regions about the ectosylvian sulci. The responses of the older kitten and adult cat cortex to a sharp auditory stimulus (clap of hands) consisted usually of a surface positive wave (50–100 microvolts) about 0.05 second (0.03–0.15 sec.) duration (fig. 2). The disappearance of the response after a certain duration of cerebral anemia, despite repeated noises of the same or greater intensity, acted as an obvious control for other vibrations set up with each noise. Frequently, the response was diphasic with a surface negative phase following a surface positive one. In some cases the response consisted of an initially surface negative diphasic wave. Metrazol was found to enhance the magnitude of the response and to increase the number of after waves. Metrazol also caused

the appearance of the auditory response in adjacent cortical regions which had previously not shown it.

Evoked cortical response and cerebral anemia. Whereas the ordinary spontaneous electrical activity in the cat (about 19 days old and older) cerebral cortex disappears within about 15 seconds after initiating complete cerebral anemia, electrical responses of the acoustic cortex to auditory

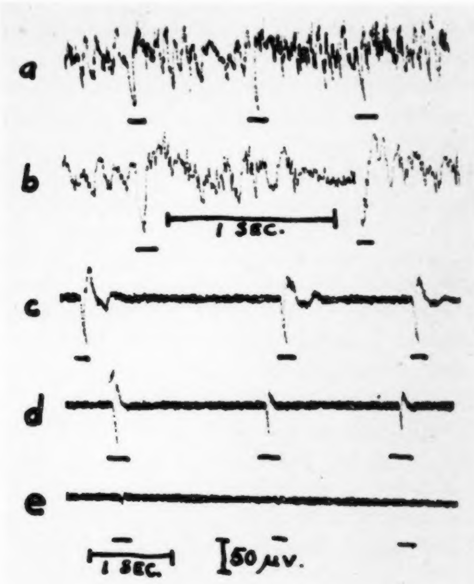

Fig. 2. Cortical acoustic response in cerebral anemia.

A. Normal spontaneous activity, 5 week old kitten cerebral cortex, from ectosylvian gyrus. Each mark underneath indicates a clap of hands. (These were marked down by hand, and the exact time of the sound cannot be told, i.e., latent period cannot be measured.) B. Same as A faster speed recording. C. Sixteen seconds after heart cut out. Spontaneous waves are already gone. D. Fifty seconds after heart out. E. Eighty seconds after heart out.

All (except B) at same speed recording (see below E).

stimuli can be evoked for as long as 50 to 100 seconds after complete cerebral anemia (fig. 2). All phases of the evoked response gradually diminish in amplitude, although sometimes an initial increase in magnitude occurs. In one case (fig. 2), a second small surface positive and negative wave which followed each initial response disappeared 45 seconds before the latter.

Discussion. These results reveal that the characteristic adult activity is not apparent in the newborn kitten. However, there seems to be a gradual increase with age in the frequency, regularity, and amplitude of the waves. Adult activity was not fully developed in 25 day old animals. A similar development of the electroencephalograph of humans has been described (5).

Since metrazol and strychnine can induce electrical activity in the cerebral cortex of the infants, the neurones in the cortex of the kitten possess the ability to produce electrical oscillations when influenced by appropriate agents. These agents may act either by causing synchronization of waves already present asynchronously in the various units or by altering the metabolism or membrane properties of the cells in such a way as to initiate oscillatory activity. The ability of the 4 day old kitten cortex to show electrical responses to acoustic stimuli, though it is comparatively lacking in spontaneous activity, indicates that there is already present in the infant functional sensory transmission to the cortex. Whether these electrical responses are post-synaptic or electrical changes from presynaptic fibers to the cortical cells cannot be determined.

The activity induced in the kitten by metrazol is somewhat different than that occurring in adult cats with or without metrazol. In the infant both the regular rhythms and the large polyphasic convulsive type of waves are slower than in the adult.

The doses of metrazol required to produce some slight change in the electrical activity in the 2 to 6 day old, 19 to 25 day old kittens and the adult cat were approximately 0.05 cc., 0.1 cc. and 0.2 cc. respectively. Since the respective weights are approximately 100, 250 and 2500 grams, it is apparent that the dose per unit of body weight is larger for the infant. The doses necessary to produce the convulsive type of spike-like waves, as well as convulsive motor activity, show a similar relationship to age, namely, about 0.1, 0.3 and 0.6 cc. of metrazol respectively. Since the ratio of the minimal convulsive dose of the infant, as compared to the adult, is about 1 to 5 while the ratio of their body weight is about 1 to 25, the infant's central nervous system is apparently less responsive to metrazol.

The results also indicate a greater resistance of the infant brain to cerebral anemia and are in accordance with the observation on revival ability of the central nervous system of puppies and adult dogs after cerebral anemia (6). It should be noted, however, that though the survival time of the 2 to 6 day old kitten was somewhat longer than in the adult that of the 19 to 25 day old kitten was not appreciably so. Kabat and Dennis (6), on the other hand, found that puppies of 49 days of age could be revived after longer periods of cerebral anemia than those which could be sustained by the adult dog. This, with our finding on the survival time of the 19 to 25 day old kitten, may indicate that revival time is not nec-

essarily a direct function of survival time. In seeking an explanation for these differences between the infant and adult brain, *in vitro* studies of their respiratory metabolism are suggestive. It has been observed that the cortex of infant rats, puppies and kittens possesses a lower metabolic rate than adults of these species (7, 8).

That the evoked cortical acoustic response can be elicited long after spontaneous electrical activity in the cortex has disappeared during complete cerebral anemia is highly interesting. It recalls the observation of Forbes and Morison (9) of evoked potentials elicited in deep barbiturate anesthesia when spontaneous waves had disappeared. However, these investigators could elicit their secondary discharge response, which resembles our evoked response, only after spontaneous activity had been abolished, while our acoustic response could be elicited in the presence of spontaneous activity as well as in its absence. On the other hand, Sugar and Gerard (10) found that spontaneous waves and evoked visual potentials in the lateral geniculate of the cat disappeared simultaneously (though later than cortical spontaneous waves) during complete cerebral anemia.

The observed evoked potential in response to an acoustic stimulus may be 1, a result of electrical changes in the cortical cells or dendrites responding to synaptic stimulation, or 2, an action potential of nerve fibers carrying impulses to the cortex, or 3, a combination of 1 and 2. The relatively long duration (about 0.05–0.15 sec.) and large size (50–100 microvolts) of the response waves as recorded from the cortical surface suggest the first possibility as the most probable one, though the second cannot be excluded. (See also the resemblance to the "deep response" obtained by Adrian (1) upon electrical stimulation of the cerebral cortex.) The sensitivity of spontaneous cortical activity to anemia (i.e., its disappearance within 15 seconds of complete anemia) has been attributed not to the nerve fibers but rather to the neurone units in the cortex (11), which presumably generate the spontaneous rhythms (12). If, then, the acoustically evoked potentials are also neuronal responses to synaptic stimulation then several interesting corollaries would follow as to 1, the relative sensitivity of synaptic transmission and spontaneous electrical activity to anemia; 2, a possible distinction between metabolism underlying the production of spontaneous activity and that underlying function in cell synaptic responses, and 3, a difference in the sensitivity of these metabolisms to anemia.

CONCLUSIONS

1. A gradual development of the frequency, regularity, and amplitude of the cortical electrograms occurs during infancy as seen in kittens 2 to 6 days old, 19 to 25 days old and adult cats.

2. Electrical activity may be induced in the comparatively silent brain

of the 2 to 6 day old kitten either by metrazol or strychnine. Acoustic stimuli can also evoke cortical responses.

3. The activity induced by metrazol in the kitten differs from that observed in the adult cat both as to central-electrical and peripheral-motor manifestations.

4. The relative dose of metrazol (per unit body weight) required to elicit an electrical or motor response is greater for the infant than the adult.

5. After sudden excision of the heart, the persistence of cerebral electrical activity is longer in the 2 to 6 day old infant than the adult. By the 19th to 25th day this difference from the adult largely disappears.

6. During complete cerebral anemia, the electrical response of the acoustic cortex to a sharp noise can be elicited for as long as 50 to 100 seconds, at a time when spontaneous electrical activity has long ceased. The possible significance of this and the above phenomena is discussed.

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THE PROTHROMBIN CONCENTRATION IN THE BLOOD OF VARIOUS SPECIES[1]

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From studies made with the quantitative method for prothrombin, which the author described in 1935, he (1) concluded that rabbit and dog blood contain several times more prothrombin than does human blood. This conclusion was further strengthened by the finding that an amount of heparin which completely inhibited the coagulation of human plasma, containing an excess of thromboplastin and an optimum concentration of calcium, was quite inadequate for preventing the clotting of rabbit plasma under identical conditions (2). It was found that the quantity of heparin which inhibited the clotting of undiluted human plasma could prevent the coagulation of rabbit plasma only when the latter was diluted with approximately four parts of prothrombin-free plasma. From this it was concluded that the prothrombin is roughly five times higher in rabbit than in human blood. Recently, however, Warner, Brinkhous and Smith (3) reported that with their quantitative method for prothrombin, which they developed contemporaneously (4) with the author's procedure, they found that the prothrombin was only slightly higher in rabbit and dog than in human plasma.

This marked discrepancy makes it desirable to investigate further the problem of the prothrombin content of various bloods, and to find if possible the reason for the differences in the results obtained by the two methods.

Experimental. *Quantitative determination of prothrombin.* The method employed in this study was the same as that which the author described for the clinical determination of prothrombin in human blood (5). The blood was obtained by venipuncture, and mixed immediately with sodium oxalate. One volume of 0.1 M sodium oxalate was added to 9 volumes of blood. It has recently been demonstrated that this amount of sodium oxalate will completely decalcify blood in less than one minute (6).

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

Thromboplastin. The most satisfactory known source of thromboplastin is rabbit brain. By dehydrating this tissue with acetone according to the author's procedure, (5), (7), a material is obtained which is not only highly active, but also uniform in potency. The preparations used in this investigation coagulated human plasma in 11 to 12 seconds and rabbit plasma in 6 seconds (when 0.1 cc. of plasma was mixed with 0.1 cc. of thromboplastin solution and 0.1 cc. of 0.025 M calcium chloride).

Thromboplastin has group rather than species specificity. Rabbit thromboplastin is specific for rabbit, dog, cat, lion, deer, horse, cow, human and many other bloods, but rather sluggishly activates the prothrombin of the guinea pig and of birds. The action of thromboplastin made from the brains of different animals and tested on the blood of these same animals is presented in table 1. It will be observed that chicken and

TABLE 1

Species specificity of thromboplastin

SOURCE OF THROMBOPLASTIN	CLOTTING TIME OF PLASMA IN SECONDS			
	Type of plasma			
	Chicken	Guinea pig	Rabbit	Man
Chicken brain	11	40	28	45
Guinea pig brain	30	16	22	35
Rabbit brain	60	24	6	11½
Human brain	240	33	10	19
Control (none added)	240	60	80	120

The clotting time was determined by mixing 0.1 cc. of plasma with 0.1 cc. of 0.025 M calcium chloride and 0.1 cc. of thromboplastin emulsion.

guinea pig thromboplastin show their greatest activity when reacting with their own prothrombins.

In this present work only the bloods of animals were studied whose prothrombin is specifically activated by rabbit thromboplastin. Most attention was given to human and rabbit blood since the first contains a relatively low prothrombin concentration, while the latter has a very high level. To obtain plasmas containing prothrombin of varying concentrations, normal oxalated plasma was diluted either with prothrombin-free plasma prepared by the author's aluminum hydroxide method (8) or with 0.85 per cent sodium chloride containing enough sodium oxalate to make a 0.01 M solution.

Results. When the clotting times of progressive dilutions of recalcified oxalated rabbit plasmas (containing excess thromboplastin) were determined, it was found that the 20 per cent solution had the same coagulation time as undiluted human plasma while a 10 per cent solution clotted at

the same rate as a 50 per cent dilution of human plasma and that the same ratio continued for further dilutions as seen in table 2. This clearly suggests that rabbit plasma contains roughly 5 times more prothrombin than is present in human plasma. Since rabbit plasma contains the highest known concentration of prothrombin, it should serve satisfactorily as the standard for expressing the prothrombin content of blood. By arbitrarily fixing the normal prothrombin content of rabbit plasma as 100, the prothrombin concentration of other bloods can be expressed. In figure 1 such a curve is presented which contains the prothrombin level of various species in terms of the normal concentration of prothrombin

TABLE 2

The relationship between the clotting time and the prothrombin concentration of rabbit and human plasma

RABBIT PLASMA PROTHROMBIN	CLOTTING TIME	HUMAN PLASMA PROTHROMBIN	CLOTTING TIME
per cent	*sec.*	*per cent*	*sec.*
100	6		
50	7		
40	8		
30	9		
25	9½		
20	11	100	11½
		80	12½
15	13		
10	15	50	15
		40	17
		30	19
5	19½	25	21½
4	26	20	25
2	37	10	39
1	55	5	65

in rabbit plasma. Rabbit and dog bloods contain the highest concentration of prothrombin while curiously the plasma of the cow has the lowest level. No explanation for these variations can be offered. It is obvious that the concentration is not influenced by the size of the animal, its dietary habits, or domestication. According to present knowledge, these variations are of no practical significance since the writer has shown that even human blood with its low prothrombin can lose almost 80 per cent of this clotting factor before a hemorrhagic state becomes manifest.

The curve expressing the relationship between the concentration of prothrombin and the clotting time (in the presence of optimal concentrations of calcium and thromboplastin) is a hyperbola. Theoretically the asymptotes of this curve are $x = 0$, $y = 0$, but practically it is rather im-

probable that a concentration of prothrombin can be found which will cause instantaneous coagulation. The curve presented in figure 1 should be considered a segment of the complete theoretical curve. In this segment the normal concentration of prothrombin in rabbit plasma is assigned as 100.

It is rather significant that if one plots the clotting times of plasma or fibrinogen using progressive dilutions of thrombin, a similar hyperbolic curve is obtained. By means of the writer's modification of Eagle's method (2) a thrombin solution can be prepared which is not only highly

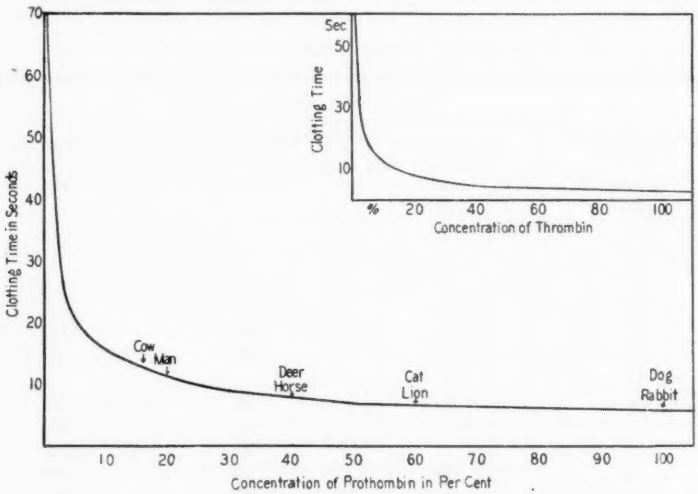

Fig. 1. The prothrombin concentration in the blood of various species. In the case of the lion and deer, determinations were done on only one specimen of blood. From preliminary work indications have been obtained that the prothrombin content of baboon blood is approximately the same as that of the human, but that the prothrombin of monkey's blood is higher.

active but is also remarkably constant in potency. When 0.1 cc. of this preparation is allowed to react with 0.2 cc. of oxalated plasma or a solution of fibrinogen, coagulation occurs in 3 seconds. By designating the concentration of this solution of thrombin 100, and determining the clotting times for a series of dilutions, the curve in the insert of figure 1 is obtained. The close similarity between the prothrombin and thrombin curves is additional evidence that the rate at which thrombin is formed is directly dependent on the concentration of prothrombin in the plasma, provided that the calcium and thromboplastin are kept constant.

Comment. From the results obtained in this study, one can conclude

that the prothrombin varies greatly in different species and that the blood of man contains only one-fifth as much prothrombin as rabbit or dog blood. Since these findings differ radically from those reported by Smith and his co-workers, it seems desirable to find the reason for this disagreement. Although it is asserted that Smith's method must remain the standard for the quantitative determination (9), certain observations are not in accord with this claim. It has not been demonstrated that the thrombin obtained in the final dilution of the 2-stage method is equal to the total that the prothrombin in the original plasma can yield. When Smith, Warner and Brinkhous improved their method, they were able to increase the units of prothrombin of normal dog blood from 200–325 to 325–400 (10). Thus, with only a small modification they increased substantially the yield of thrombin from the same amount of prothrombin.

If prothrombin is a pre-enzyme closely associated with plasma proteins, it is problematic whether such a substance can be diluted several hundred times without undergoing a certain amount of degeneration especially because of the disturbance of the protective colloids. A probable illustration of this is brought out in the determination of the prothrombin in the blood of newborn infants. Brinkhous, Smith and Warner (11) found that the prothrombin concentration was below 50 per cent of normal up to the age of two months and did not reach the adult level until the ninth month. The writer with Grossman (12) found, on the contrary, that the prothrombin was normal in infants except for a few days after birth. This short period of prothrombinopenia corresponds to the time when bleeding is frequently observed, and it can be prevented by means of vitamin K. Smith and his associates (13) believe that the prothrombin of the newborn is converted more readily to thrombin to compensate for the deficiency in quantity. While it is known that the speed of the conversion of prothrombin is influenced by the concentrations of calcium, thromboplastin, heparin and perhaps other substances, no direct evidence can be found that prothrombin itself can alter its convertibility. If the prothrombin is qualitatively different in infants, one must conclude that vitamin K increases the convertibility rather than the quantity of prothrombin. This, however, is not in accord with the results obtained using vitamin K in the prothrombinopenia of jaundice. In the latter condition all the results obtained both by Smith's and by the writer's method clearly indicate that vitamin K restores the prothrombin content of the plasma and does not influence the convertibility. It seems very likely therefore that the prothrombin of infants is qualitatively normal, but the plasma varying as it does from the adult, may not yield on dilution as much thrombin per unit of prothrombin as does adult blood. Likewise, it is probable that when the plasma of various species is diluted several hundred fold, and then treated with calcium and thromboplastin, the resulting mixture will represent an equilibrium of

thrombin, degenerated prothrombin and metathrombin rather than a solution of pure thrombin.

SUMMARY

By means of the method developed by the writer for the quantitative determination of prothrombin, it has been found that the concentration of this factor varies greatly in different species. If the prothrombin level of normal rabbit plasma is set at 100, the concentration found in various species is as follows: dog 100; cat 60; lion 60; horse 40; man 20; cow 16.

The probable reason why the 2-stage method of Smith for the determination of prothrombin fails to show this marked variation in prothrombin content of various bloods is discussed.

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THE EFFECT OF DENERVATION UPON THE RESPONSE TO ADRENALIN IN THE ISOLATED FISH SCALE MELANOPHORE

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Adrenalin brings about rapid and complete concentration of melanophore pigment in teleosts either when injected or applied directly to the surface of the body. Microscopic examination reveals the cell in a typical punctate condition with the pigment solidly concentrated in the center. With the exception of sympathetic nerve stimulation no other agent is capable of producing so rapid and thorough a response. This fact has long been familiar to those working in the field of animal color changes. It has led to the view that the action of adrenalin is physiologically significant in melanophore responses and is probably involved in the normal paling of a fish upon a light background. This position is greatly strengthened when the rôle of the sympathetic nervous system in governing teleost color changes is considered. Control of melanophore activity in the bony fishes by the sympathetic nervous system has been thoroughly established by the work of Pouchet (1876), v. Frisch (1911) and many others.

Whether adrenalin plays in teleosts the same part in transmitting sympathetic nerve impulses from nerve ending to effector organ that it does in the higher vertebrates has not yet been demonstrated. It seems reasonable to assume that it does. If so it explains the great sensitivity of the melanophore to this substance. In the higher vertebrates the sensitivity of the effector organ to adrenalin is actually increased by denervation and subsequent degeneration of the nerve ending. This was first demonstrated by Meltzer and Auer (1904) and later by Cannon and Rosenblueth (1932) in the nictitating membrane of the cat. This bit of smooth muscle responds much more vigorously and exhibits a lower threshold to adrenalin after degeneration of the myo-neural junction than before. A similar phenomenon in the isolated scale melanophores of fishes would be of considerable theoretical interest.

That such a reaction does occur in the fish melanophore can be demonstrated. Scales from the common Tautog (Tautoga onitis) of the

Atlantic seaboard were selected for the experiment. Denervation of the scale was done in the following manner, the procedure being based upon a method devised for transplanting fish scales by Mori (1931) and Goodrich and Nichols (1933). A trunk scale was carefully pulled out of its scale pocket with a forceps, obviously severing all of its connections with the nervous and circulatory systems. The scale was then slipped back into the pocket and left there undisturbed. In a vast majority of cases the scales healed back in place, quickly established new circulatory connections and maintained themselves in a healthy condition. Nervous regeneration was characteristically slower, as shown by the failure of the scales to participate for some time in the normal color changes shown by the animal. Placed on a light background an operated fish would show dark splotches on an otherwise pale body due to patches of scales in which the denervated melanophores did not respond.

While release from central nervous control is immediate, independence of nervous influences is not. Even after removal of the scale from the body it still contains the severed distal ends of the nerve fibers and the nerve endings. The nerve twigs are only a millimeter or so in length and degenerate completely in about a day. Only when this occurs can the melanophore be said to be free from any nervous control. Once affected, however, any response exhibited by the melanophores of such a scale must be free of any nervous mediation whatsoever. The behavior of such preparations has previously been discussed (Smith, 1939). Indications are that regeneration of the nervous elements in the scale occurs in about two weeks. After this time the melanophores once more take part in the normal color responses of the fish to background, or concentrate when the pigment-motor center in the medulla is electrically stimulated.

The responses of these operated scale melanophores to adrenalin were studied during the period of denervation and compared to those in normal unoperated scales and those in scales showing recovery from denervation. The response was a simple one to obtain. The scale was removed from the side of the trunk and placed in a suitable quantity of balanced salt solution consisting of 6 vols. of N/5 NaCl, 1 vol. N/5 KCl, and 0.35 vol. N/5 $CaCl_2$. To this enough adrenalin chloride was added to make the adrenalin strength of the solution $\frac{1}{100,000}$. The scale melanophores exposed to this solution, either normal or denervated, showed the typical response to adrenalin, a rapid concentration of their pigment.

The speed of the response was measured photoelectrically by a method previously described (Smith, 1936). In brief it was as follows. The amount of light transmitted through an isolated scale was measured, the amount being a function among other things of the degree of melanin dispersion within the scale melanophores. As the melanophores concen-

trated their pigment more light passed through the scale with a consequent deflection of the galvanometer attached to the photocell. Thus galvanometer deflection could be used as a sign of pigment movement.

The galvanometer deflections resulting from the pigment concentration produced by the action of adrenalin on each of ten different normal scales were recorded, the average deflection determined and plotted as shown in figure 1. Several scales must be averaged because of variations in their size, thickness and number of melanophores. Similarly the concentration of the melanophores in six scales denervated for four days and three scales denervated for six days was averaged and plotted. Finally, the results obtained from six scales thirteen days after the operation were treated in the same way. All four curves were plotted on the same graph as

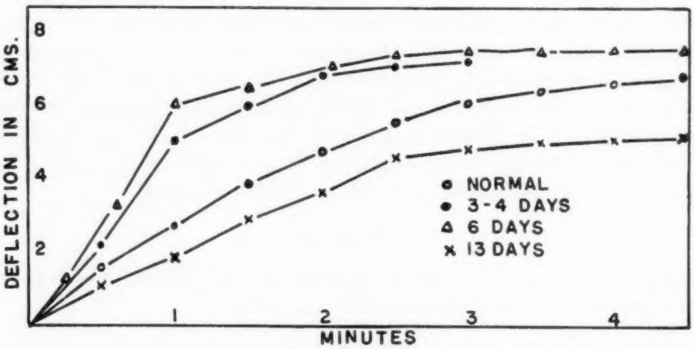

Fig. 1. Responses to $\frac{1}{100000}$ adrenalin in isolated scales of the Tautog, both normal and denervated. Photo-electric recording. Increase in galvanometer deflection indicates pigment concentration.

shown in figure 1. From this it was clear that the scale melanophores four to six days after the operation concentrated their pigment about twice as rapidly as did those in normal scales. Thirteen days after the operation the speed of the response was again the same as in the normal scales, indicating regeneration of the nervous elements lost as a result of the original operation. Thus denervation and subsequent degeneration of the chromo-neural junction resulted in an increased sensitivity on the part of Tautog scale melanophores to adrenalin. This established an essential similarity between the responses to adrenalin in fish melanophores and in the effectors of higher vertebrates where adrenalin is known to act as the mediator between the sympathetic nerve endings and the responding organ. This does not of course prove that adrenalin so acts for teleost melanophores, although it does make it appear more likely.

Aside from any possible theoretical significance these results fully corrob-

orate the demonstration by Parker (1934) that adrenalin acts directly upon melanophores. He observed the familiar response to adrenalin in the tail melanophores of Fundulus after sectioning the nerves to the region involved and allowing ample time for the degeneration of distal nerve fibers and nerve endings. Prior to this demonstration Giersberg (1930) and others maintained that adrenalin acted upon fish melanophores only by way of the nerve ending and was incapable of affecting the cell directly.

The difference in timing between the responses of normal and denervated melanophores to adrenalin serves to explain a puzzling reaction observed some years ago in Phoxinus (Smith, 1931). Certain areas on the head of this fish were denervated by sectioning the ophthalmic nerve. Remarkably enough, if such operated fishes were placed upon a black background with skins uniformly dark and then subjected to a sudden frightening stimulus such as a sharp tap on the side of the aquarium the operated areas suddenly paled although the rest of the body remained black. At the time adrenalin release seemed the likely explanation for this reaction but it was difficult to see why the entire body was not affected. In the light of the increased sensitivity to adrenalin on the part of denervated melanophores the reaction is now easily explained.

SUMMARY

Isolated scale melanophores of the fish Tautog respond more rapidly to adrenalin after denervation than they do before.

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THE EFFECT OF PRIMING DOSES OF DESOXYCORTICOSTERONE ACETATE IN PREVENTING CIRCULATORY FAILURE AND SHOCK IN THE ADRENALECTOMIZED DOG

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Active vigorous adrenalectomized dogs kept in normal condition by daily injections of maintenance doses of cortical extract are extremely susceptible to any procedure which throws a strain upon the peripheral circulation. Trauma to muscle masses, intestinal manipulation, intravenous injections of massive doses of epinephrine, or intraperitoneal injection of isotonic glucose invariably induce circulatory collapse and shock within 5 to 15 hours. The same degree of trauma or quantities of injected epinephrine and glucose cause no untoward symptoms in the animal possessing adrenal glands. Likewise, circulatory failure does not develop if the adrenalectomized dog is primed with large doses of cortical extract just previous to the shock inducing procedures. It has been demonstrated, moreover, that if the animals are permitted to develop circulatory failure and shock, they can be readily revived and restored to normal by injections of adequate amounts of cortical extract (1, 2).

Since synthetic D.C.A. (desoxycorticosterone acetate) will maintain adrenalectomized dogs and rats (3–7) and is said to have a prophylactic action in human surgical shock and analogous conditions where circulatory failure is a prominent symptom (8–10), it was considered worth while to study its effect upon experimentally induced circulatory failure in the adrenalectomized dog.

Methods. The adrenalectomized dogs employed had been operated for three months to one year before use, and kept in normal health by daily injections of maintenance doses of cortical extract. These dogs were active, vigorous, and at peak weight. Earlier studies by two of the writers (2) have demonstrated that in this type of animal the arterial pressure, serum electrolyte concentrations, blood sugar level, blood urea nitrogen concentration, water balance and renal excretion of electrolytes

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are normal as compared with nonadrenalectomized dogs maintained under similar laboratory and dietary conditions.

The method used for inducing circulatory failure and shock were: 1, the intraperitoneal injection of an isotonic glucose solution; 2, the injection of large amounts of epinephrine; 3, trauma to muscle masses, and 4, manipulation of the small intestine.

A dose of 60 cc. per kgm. body weight of a 5.5 per cent glucose solution was injected intraperitoneally without use of an anesthetic to induce glucose shock. Since extensive experimentation has shown that the circulatory failure in the adrenalectomized animal occurs independently of the presence or absence of the peritoneal fluid (11–12), paracentesis was not performed.

In the epinephrine experiments, a 1-5000 solution in 0.9 per cent saline of adrenalin hydrochloride (Parke, Davis and Co.) was injected into the saphenous vein at a rate of 0.5 to 0.8 cc. per minute, depending on the condition of the animal. When retching or vomiting occurred, the injection was stopped and the animal allowed a rest until the symptoms disappeared. No anesthetic was employed.

In the muscle trauma experiments, the dogs were deeply anesthetized with ether, and the thigh muscles struck a definite number of blows with a wooden mallet. The strength of the blows was just sufficient to severely bruise the muscles without producing bone fractures.

The animals used for intestinal manipulation were anesthetized with ether and, using strict asepsis, a three-inch slit made in the abdominal wall. A short segment of the small intestine was then lifted through the incision and gently stripped through the fingers, only a few inches of intestine being exposed at any time. The stripping was continued in this manner over the entire length of the small intestine for thirty minutes. The wound was then sutured and bandaged, and the dog allowed to recover from the anesthetic.

The arterial pressures were determined without anesthesia by the direct intra-arterial needle puncture method (13). The readings represent mean pressures. An initial reading was taken shortly before the experiment was begun, and all further pressures taken after recovery from the anesthetic. Since it has been shown (2) that, exclusive of the intraperitoneal glucose experiments, the serum Na, Cl, and K concentrations remain unchanged in the shock induced by these procedures, electrolyte studies were not made.

The dosage of D.C.A.[2] varied in the earlier experiments, but in the later work 15 mgm. were given in three doses of 5 mgm. each by intramuscular injection. The usual time interval for these injections was 18, 12 and 2

[2] We are indebted to the Ciba Pharmaceutical Products, Inc. for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

hours before the experiment. All cortical extract was withdrawn 36 hours before the experiment. D.C.A. in oil can be used only for priming, i.e., as a prophylactic agent, since absorption after either subcutaneous or intramuscular injection is too slow to be of value once shock has developed.

I. *Intraperitoneal glucose injections.* Six adrenalectomized dogs were primed with D.C.A. and subjected to intraperitoneal glucose injections. Typical protocols are shown in table 1. None of these animals showed symptoms of circulatory failure or shock. In contrast to the behavior of the D.C.A. treated dog is that of the nonprimed animal receiving the same amount of glucose (dogs 5, 6, table 1). These animals exhibit circulatory collapse within 2 to 6 hours after the glucose administration, and will die within a few hours if left untreated.

II. *Epinephrine injections.* A dose of 0.4 mgm. per kgm. body weight of epinephrine will produce fatal shock in the adrenalectomized animal maintained on cortical extract, while one of at least 0.7 mgm. per kgm. body weight is required to produce shock in either the intact dog or the adrenalectomized dog receiving priming doses of extract. Epinephrine doses of 0.4 to 0.7 mgm. per kgm. were given seven adrenalectomized dogs, five being primed with D.C.A. and two untreated. Representative data are given in table 2. It is clear that the dog primed with D.C.A. is resistant to the amount of epinephrine which will produce fatal shock in the nontreated animal. It is possible that it does not afford the same degree of protection as does cortical extract, for a D.C.A. primed animal receiving 0.7 mgm. per kgm. body weight (dog 4, table 2) did not show the expected spontaneous pressure rise. Although this dosage was within the lower limits of the amount required to produce shock in the intact dog, cortical extract will usually protect the animal against even greater amounts of epinephrine. Cortical extract was used to save the life of this animal.

III. *Muscle trauma.* Typical protocols from the study of seven adrenalectomized dogs primed with D.C.A. and subjected to muscle trauma are given in table 3. In striking contrast to the responses of dogs 1 to 4, which were primed previous to traumatization, and in which symptoms of shock did not develop, are those of dogs 5 and 6, not given treatment, in which death followed within nine hours. The writers, in numerous experiments, have never observed an adrenalectomized dog receiving only maintenance doses of extract fail to develop circulatory collapse within a few hours after the same degree of trauma. Animals primed with cortical extract likewise show no symptoms.

IV. *Intestinal stripping.* Pertinent data from the study of eight adrenalectomized dogs after intestinal manipulation are given in table 4. Circulatory failure and shock occurred in all cases. The D.C.A. priming apparently had no prophylactic action—a result in striking contrast to its protection against shock in the other experiments. We have no ade-

quate explanation for its failure here. Negative results with D.C.A. after intestinal manipulation have also been reported by Selye and co-

TABLE 1

Action of desoxycorticosterone acetate on adrenalectomized dogs receiving intraperitoneal injections of isotonic glucose

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	REMARKS
		Dog 1. 10.2 kgm. Primed with D.C.A.		
		mm. Hg		
6/19	10:30 a.m.	98	72	Injected with glucose
	3:30 p.m.	73	140	No symptoms, active
	8:30 p.m.	68	140	No symptoms
6/20	4:00 a.m.	95	140	Normal, hungry
		Dog 2. 6.5 kgm. Primed with D.C.A.		
6/25	10:30 a.m.	88*	84	Injected with glucose
	3:30 p.m.	70	124	No symptoms
	8:30 p.m.	69	154	No symptoms, active
6/26	9:30 a.m.	89	92	Normal
		Dog 3. 9.1 kgm. Primed with D.C.A.		
7/1	10:00 a.m.	116	112	Injected with glucose
	3:00 p.m.	93	172	No symptoms
	11:00 p.m.	79	164	Active, no symptoms
7/2	10:00 a.m.	113	140	Normal
		Dog 4. 10.9 kgm. Primed with D.C.A.		
6/25	10:00 a.m.	104	96	Injected with glucose
	3:00 p.m.	75	140	No symptoms
	8:30 p.m.	72	152	No symptoms
6/26	9:30 a.m.	89	128	Appears normal. Experiment discontinued
		Dog 5. 9.3 kgm. No treatment, control		
5/16	10:30 a.m.	100	120	Injected with glucose
	4:30 p.m.	48	140	Very weak, walks with difficulty
	8:00 p.m.	38	140	Complete collapse. Revived with extract
		Dog 6. 8.9 kgm. No treatment, control		
5/18	11:00 a.m.	108	100	Injected with glucose
	7:30 p.m.	68	188	Depressed, inactive
	11:30 p.m.	46	140	In collapse. Revived with extract

* Animal had not received cortical extract for two days previous to priming with D.C.A.

workers (14) with intact rats, and by Weil and associates (15) using normal rabbits. They report that while cortical extract and corticosterone re-

duce the mortality from shock, D.C.A. was inactive and in some cases actually proved to be harmful. That cortical extract will revive the animals used here is illustrated by dog 1, in which extract was used to save the animal's life after 20 mgm. D.C.A. failed to protect against shock.

TABLE 2

Protective action of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following massive injections of epinephrine in adrenalectomized dogs

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	REMARKS
Dog 1. 9.4 kgm. Given 0.6 mgm. epinephrine/kgm. 15 mgm. D.C.A.				
		mm. Hg		
10/8	10:00 a.m.	102	116	Injection started, finished at 10:50 a.m.
	12:00 m.	88	168	Depressed but no signs of weakness
	1:00 p.m.	94	152	Active, vigorous
	3:00 p.m.	104	152	Normal
Dog 2. 10.4 kgm. Given 0.5 mgm. epinephrine/kgm. 15 mgm. D.C.A.				
7/18	10:10 a.m.	92	80	Injection started, finished at 11:30 a.m.
	11:35 a.m.	53	160	Markedly depressed
	4:30 p.m.	80	120	Active, vigorous
7/19	10:30 a.m.	83*	76	Ate full ration, active
Dog 3. 10.8 kgm. Given 0.5 mgm. epinephrine/kgm. 15 mgm. D.C.A.				
7/18	2:20 p.m.	94	80	Injection started, finished at 3:30 p.m.
	3:40 p.m.	68	172	Depressed, inactive
	9:30 p.m.	89*	100	Active, vigorous
Dog 4. 11.1 kgm. Given 0.7 mgm. epinephrine/kgm. 15 mgm. D.C.A.				
10/14	9:40 a.m.	110	92	Injection started, finished at 10:50 a.m.
	11:00 a.m.	92	152	Depressed
	1:00 p.m.	72	160	Lethargic, depressed
	5:10 p.m.	58	168	Very weak. Given 3 cc./kgm. cortical extract
	11:00 p.m.	86*	140	Marked improvement, active, bright, ate food
Dog 5. 9.8 kgm. Given 0.4 mgm. epinephrine/kgm. No treatment				
6/5	9:30 a.m.	103	80	Injection started, finished at 10:20 a.m.
	3:20 p.m.	89	175	Inactive, depressed
	10:40 p.m.	50	180	Profound shock
	11:40 p.m.	36	200	Died 20 minutes later

* Experiment discontinued.

Four of the eight dogs exhibited unusual symptoms which were quite alien to those of shock. There seemed a correlation between the amount of D.C.A. given, the time allowed for absorption, and the appearance of

TABLE 3

Effectiveness of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following muscle trauma in adrenalectomized dogs

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	REMARKS
Dog 1. 12.6 kgm. 30 mgm. D.C.A.				
		mm. Hg		
4/22	4:00 p.m.	106	120	Primed with 10 mgm. D.C.A. at 10:30 a.m., 1:30 p.m. and 4:30 p.m. 80 blows to right hind leg muscles at 4:30 p.m.
	8:30 p.m.	99	120	
	11:30 p.m.	92	147	No symptoms. Leg greatly swollen. Dog active, bright
4/23	10:30 a.m.	92	130	Hops about in cage. Ate full ration
	4:30 p.m.	96	120	Normal
Dog 2. 10.7 kgm. 15 mgm. D.C.A.				
7/10				Primed with 5 mgm. D.C.A. at 5 p.m., 11 p.m. and at 8 a.m. on 7/11
7/11	10:00 a.m.	99	60	Given 100 blows to right hind leg muscles
	4:30 p.m.	89	128	No symptoms. Leg swollen, hops about in cage. Bright
	11:30 p.m.	94	160	Active and vigorous. Ate full ration eagerly
7/12	8:30 a.m.	96	88	Leg still swollen. Active, vigorous
Dog 3. 11.1 kgm. 15 mgm. D.C.A.				
7/11				Primed with 5 mgm. D.C.A. at 5 p.m., 11 p.m. and at 8 a.m. on 7/12
7/12	10:00 a.m.	86*	80	Given 95 blows to right hind leg muscles
	3:30 p.m.	72	142	Leg swollen. No symptoms, dog quiet
	11:30 p.m.	64	140	Active, ate full ration eagerly
7/13	9:30 a.m.	72	140	Leg still swollen. Active, alert
7/14	9:30 a.m.	86	90	Normal
Dog 4. 11.4 kgm. 5 mgm. D.C.A.				
7/14				Primed with 5 mgm. D.C.A. at 11 p.m.
7/15	9:30 a.m.	108	120	Given 110 blows to right hind leg muscle
	2:30 p m.	108	152	No symptoms. Leg swollen
	11:30 p.m.	103	160	Ate full ration eagerly
7/16	9:30 a.m.	105	120	Normal
Dog 5. 10.5 kgm. No treatment				
8/30	12:30 p.m.	101	85	Given 97 blows to right hind leg muscles
	7:00 p.m.	83	174	Depressed, dull. Leg greatly swollen
	9:30 p.m.	35	173	In collapse. Died at 10:15 p.m.
Dog 6. 14.0 kgm. No treatment				
8/30	10:00 a.m.	100	70	Given 100 blows to right hind leg muscles
	12:15 p.m.	72	98	Depressed
	2:40 p.m.	56	140	Weak, symptoms of shock
	4:15 p.m.	42	176	Prostrate
	7:10 p.m.	36	182	Died at 8:00 p.m.

* Animal had low pressure before adrenals removed.

TABLE 4

Non-effectiveness of priming doses of desoxycorticosterone acetate in preventing circulatory failure and shock following intestinal manipulation in adrenalectomized dogs

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	REMARKS
		Dog 1. 10.0 kgm. 30 mgm. D.C.A.		
		mm. Hg		
6/2				Given 10 mgm. D.C.A. at 9:30 a.m. and at 9:30 a.m. and 12:15 p.m. on 6/3
6/3	11:45 a.m.	104	100	Given 30′ intestinal stripping
	3:15 p.m.	52	130	Prostrate
	8:15 p.m.	34	140	Still in collapse. Unusual symptoms*
	11:00 p.m.	36	140	Given 30 cc. cortical extract
6/4	10:00 a.m.	70	140	Bright and alert. Ate full ration. 30 cc. extract
6/5	10:00 a.m.	98	100	Normal
		Dog 2. 12.0 kgm. 20 mgm. D.C.A.		
6/2				Given 10 mgm. D.C.A. at 9:30 a.m. on 6/2 and 6/3
6/3	2:15 p.m.	106	90	Given 30′ intestinal stripping
	4:45 p.m.	102	120	Active, no symptoms
	7:45 p.m.	68	160	Depressed, unusual symptoms*
	10:45 p.m.	33		Complete collapse. Died at 11 p.m.
		Dog 3. 10.1 kgm. 15 mgm. D.C.A.		
7/1				Given 5 mgm. D.C.A. at 5 p.m., 11 p.m. and 8 a.m. on 7/2
7/2	9:45 a.m.	105	80	Given 30′ intestinal stripping
	1:30 p.m.	109	120	Active, no symptoms
	4:30 p.m.	85	160	No symptoms
	9:00 p.m.	68	160	Depressed, lethargic
	11:00 p.m.	56	168	Bloody diarrhea, in collapse, given 30 cc. cortical extract
7/3	7:30 a.m.	48	168	Little improvement. Given 30 cc. extract
	3:30 p.m.	73	148	Bright, active, ate food
7/4	9:30 a.m.	100	90	Normal
		Dog 4. 6.6 kgm. 15 mgm. D.C.A.		
6/30				Given 5 mgm. D.C.A. at 5 p.m., 11 p.m. and 8 a.m. on 7/1
7/1	10:15 a.m.	99	74	Given 30′ intestinal stripping
	12:45 p.m.	87	68	Bloody stools, depressed
	3:45 p.m.	45	180	Very weak
	4:30 p.m.	34	182	Collapse. Died within an hour

* See text.

TABLE 4—*Concluded*

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	REMARKS
		Dog 5. 10.4 kgm. No treatment		
		mm. Hg		
5/16	10:30 a.m.	110	132	Given 30′ intestinal stripping
	12:30 p.m.	82	200	Depressed
	4:40 p.m.	42	180	Collapse. Died at 6:45 p.m.
		Dog 6. 11.1 kgm. No treatment		
5/16	10:30 a.m.	110	132	Given 30′ intestinal stripping
	1:15 p.m.	85	152	No symptoms
	4:15 p.m.	45	152	Very weak, shock
	8:15 p.m.	32		Died on table

the symptoms. They have not been observed in any of the experimental procedures other than intestinal stripping. Within a few hours after the intestines were stripped, an excitability with muscular twitching developed. Eventually convulsive seizures ensued, with rigid extension of the forelegs, generalized muscle tremors, and opisthotonus. The convulsive state was followed by extreme muscular weakness with flaccidity of the neck muscles. The dog was in semi-conscious state throughout. These symptoms resemble those reported by Kuhlmann and associates (16) in intact dogs after excessively large doses of D.C.A. had been given for a relatively long period of time. These authors ascribed the symptoms to an abnormally low serum potassium level.[3]

Discussion. The writers have advocated for some years that an important function of the adrenal cortex lies in its "pressor" action at the vascular periphery. This is not to imply that the hormone acts directly to raise the blood pressure, as does a true pressor drug, but only that the secretion of the adrenal cortex is necessary in some way for the maintenance of the normal ability of the periphery to cope with a vascular strain.

One of the cardinal features of adrenal insufficiency in the dog is the slow but steady decline in arterial pressure, a fall which appears before the blood picture has been appreciably altered. By the time the animal is showing marked symptoms of insufficiency, the pressure is at "shock" levels. Even when the dog is maintained in excellent health by adequate doses of extract, a sudden stress on the vascular periphery (e.g., a prolonged vasoconstriction) leads to a slow but progressive fall in blood

[3] Further experimentation has shown that corticosterone adequately protects against circulatory failure induced by intestinal stripping. D.C.A. affords no protection against circulatory collapse resulting from one other type of trauma, while cortical extract is highly effective.

pressure and eventual circulatory collapse within 6 to 10 hours. We have observed no instance when the pressure could be spontaneously raised and maintained after this progressive decline had started. Adequate amounts of cortical extract will interrupt the fall, and restore the circulation to normal, even though the pressure has reached "shock" levels. If the adrenalectomized animal has been previously primed with extract, the resistance to peripheral vascular strain is as marked as in the intact dog. It should be borne in mind that the only change manifest in this experimentally induced shock is the low blood pressure, for serum electrolyte concentrations, blood glucose, etc., remain normal. Hence the normal picture of adrenal insufficiency has not been reproduced, the procedures used sufficing only to throw into exaggerated light the inability of the adrenalectomized dog to make peripheral vascular capacity adjustments in the absence of hormone and the restoration of this ability when hormone is given. The evidence would indicate a fundamental role of the adrenal cortical hormone in maintenance of the functional integrity of some part of the peripheral vascular apparatus.

Since D.C.A. will protect the adrenalectomized animal against shock after muscle trauma, epinephrine injections, and intraperitoneal glucose injections, it, too, presumably has an action on the peripheral vasculature. It may not afford a protection equal to that of cortical extract. The failure to protect against shock after intestinal stripping indicates that at least in this particular experiment D.C.A. is not identical with cortical extract.

SUMMARY

1. Desoxycorticosterone acetate successfully protected the adrenalectomized dog against circulatory collapse and shock following the intraperitoneal injection of an isotonic glucose solution, the injection of large amounts of epinephrine, and trauma to muscle masses.

2. In contrast, no evidence of an effective protection was found against the shock following intestinal stripping.

3. When large doses of desoxycorticosterone acetate were used in the intestinal stripping experiments, unusual symptoms appeared which the writers have never before observed accompanying this type of shock.

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THE INFLUENCE OF VITAMIN E-DEFICIENCY ON THE ENDOCRINE GLANDS OF RATS, PARTICULARLY ON THE GONADOTROPIC HORMONE CONTENT OF THE PITUITARY GLAND[1]

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Studies concerning the influence of vitamin E-deficiency of rats upon the gonadotropic hormone content of the pituitary gland are conflicting. It has been reported that there is an increase in the male (1, 2), and no effect (1), an increase (3), and a decrease (4, 2) in the female. The present study was undertaken to reinvestigate the problem upon a large number of assay animals. It was also desired to determine whether each component of the gonadotropic complex (follicle stimulating and luteinizing hormone) was influenced quantitatively in instances where changes in the gonadotropic hormone content of the pituitary gland were produced. Complete autopsy data on all the endocrine glands of large groups of normal, vitamin E-deficient, and castrate male and female rats were taken. The gland weights of these rats are included in this report.

Materials and methods. The diet used to produce the vitamin E-deficiency was the standard E-low diet[2] (diet 427) of Evans, et al. (5, 6). Rats from our own colony, which are of the Sprague-Dawley strain, were used in all the experiments. They were placed on the E-deficient diet at one month of age and maintained on that diet until autopsy, which was performed at 4, 7, 13 and 16 months of age. The animals received the diet ad libitum. Groups of rats were gonadectomized at one month of age and kept until 4, 7 and 13 months of age before autopsy was performed. Normal animals of the same age were sacrificed with each group, so that it is possible to compare data from normal, E-deficient and castrate males at the ages indicated. Comparisons of all three types of females were made only at 7 months of age. The normal and castrate animals were fed with Wayne dog chow.

[1] This work was supported by a grant from the Wisconsin Alumni Research Foundation. Assistance was also furnished by the personnel of the W.P.A. Official Project no. 65-1-53-2349.

[2] We are greatly indebted to Mr. E. A. Webb of the Anheuser-Busch Co., St. Louis, Mo., for furnishing the dried brewers' yeast used in the diet.

The animals were killed with ether and then autopsied. The pituitary gland was immediately removed, weighed and crushed between two glass plates. The pituitary tissue was allowed to dry at room temperature for 3 to 5 days, after which it was removed from the plates with a razor blade, weighed and stored in individual vials in a desiccator. Data will be presented to show that there is no appreciable loss of potency of glands stored in this manner for as long as 16 months.

Subsequent to the removal of the pituitary gland, the remaining endocrine glands were removed and weighed to the nearest milligram.

The pituitary glands of the different groups were assayed after all the glands had been collected. At this time they had been stored for one-half to 15 months. Female rats of the Sprague-Dawley strain, weighing from 34 to 37 grams, were brought into the laboratory at 21 days of age. On the 25th day of life they were hypophysectomized and the following day injections of the pituitary material were begun. The pituitary powder was easily made into a fine suspension by flushing the material back and forth into a syringe through a 23 gauge hypodermic needle. The injections were made subcutaneously twice daily for 4½ days with autopsy on the morning of the sixth day, at which time the ovaries, adrenals and thyroids were removed, examined and weighed. Three animals were injected with each dosage of pituitary powder, thus a total of 162 hypophysectomized animals were used in the assays.

Results and discussion. *Endocrine gland weights of male rats.* An analysis of the autopsy data of the 311 male rats included in table 1 shows the following:

1. The testes of the E-deficient males weighed less than one-half as much as the testes of normal males. The atrophy was as marked after 3 months of E-deficiency as after 12 or 15 months, when judged on a weight basis. Histological sections, however, showed that the degeneration of the seminiferous epithelium was not complete after 3 months, but was complete after 6, 12, and 15 months. Supplementing the diet with wheat germ oil[3] (0.2 cc. per day for the first 3 months and 0.3 cc. per day for the following 3 months) maintained normal testis weight and a normal quantity of sperm in the seminiferous tubules. There was no detectable difference in the amount of interstitial tissue in the testes of the E-deficient and normal rats at any age.

2. The weight of the seminal vesicles and prostate of the E-deficient males, when analyzed statistically, was significantly greater than that of the corresponding glands of normal rats at 4 months of age. The weight difference of these organs at 7 and 13 months was not statistically significant. Supplementing the diet with wheat germ oil gave accessory glands

[3] We wish to thank Mr. A. E. Pacini of the Archer-Daniels-Midland Co., Minneapolis, Minn., for the generous supply of wheat germ oil.

that were significantly heavier than those of both normal and E-deficient animals. This response may indicate that wheat germ oil possesses androgenic or gonadotropic properties. Verzar (7) claimed that injections of wheat germ oil induced precocious development of the uterus of rats, but Diakov and Krizenecky (8) were unable to confirm this.

TABLE 1

Autopsy data of normal, vitamin E-deficient and castrate male rats

TREATMENT	AGE AT AUTOPSY	NO. RATS	BODY WT.*	GONADS	SEM. VESC.	PROSTATE	ADRENALS	THYMUS	THYROID	PITUIT.
	mo.									
Normal	4	26	245 ±4.88	3021 ±50.78	654 ±30.80	602 ±26.85	36 ±1.65	340 ±18.29	12 ±0.32	6.9 ±0.14
E-def. 3 mo.	4	37	310 ±6.17	1246 ±25.27	861 ±22.15	731 ±19.98	43 ±0.99	361 ±10.00	15 ±0.36	8.4 ±0.17
Castr. 3 mo.	4	33	256 ±4.28		10 ±0.31	18 ±0.49	47 ±1.19	361 ±16.20	13 ±0.37	12.3 ±0.24
Normal	7	31	326 ±7.80	2861 ±83.20	688 ±45.50	683 ±30.80	34 ±0.80	183 ±10.80	15 ±0.44	7.3 ±0.01
E-def. 6 mo.	7	30	313 ±7.05	1240 ±32.54	780 ±47.18	727 ±48.55	41 ±1.08	300 ±12.70	32 ±0.85	9.0 ±0.25
E-def. + wht. germ oil** 6 mo.	7	32	392 ±6.72	2977 ±50.56	993 ±43.31	941 ±31.55	47 ±1.46	279 ±8.77	20 ±0.50	9.1 ±0.20
Castr. 6 mo.	7	36	324 ±7.41		37 ±2.03	44 ±2.03	45 ±1.46	283 ±13.60	17 ±0.52	14.8 ±0.36
Normal	13	23	370 ±9.83	3060 ±58.95	973 ±57.11	1009 ±38.95	41 ±1.50	202 ±16.28	17 ±0.64	9.1 ±0.27
E-def. 12 mo.	13	28	307 ±8.52	1222 ±52.53	1067 ±61.02	902 ±45.11	56 ±2.05	174 ±10.03	25 ±0.65	9.4 ±0.25
Castr. 12 mo.	13	28	340 ±9.79		15 ±0.69	19 ±0.68	51 ±1.34	179 ±42.59	17 ±0.70	15.7 ±0.52
E-def. 15 mo.	16	7	288 ±19.73	1338 ±125.83	682 ±125.07	709 ±108.71	65 ±2.30	137 ±24.58	26 ±1.85	10.8 ±0.56

* The body weight is in grams; all other weights are in milligrams. The standard error of the mean of each weight is also given.

** Each rat received 0.2 cc. of wheat germ oil per day from a syringe for the first three months and 0.3 cc. per day for the following three months.

The weight of the seminal vesicles and prostate after 15 months of E-deficiency was 36 and 21 per cent respectively, less than the weight of these glands after 12 months. This decrease was correlated with the condition of the animals at this age, for the muscular dystrophy and weakness characteristic of long periods of E-deficiency (9) were very marked. It is doubtful, however, whether this atrophy can be attributed solely,

if at all, to the E-deficiency, for it has been reported that inanition induces an atrophy of the accessory glands (10). Kudrjaschov (11) has claimed that E-deficiency *per se* produces atrophic accessory glands.

3. The adrenal glands of the E-deficient males were intermediate in weight between the adrenals of normals and castrates at all ages except 13 months. At the latter age they were heavier than those of both normals and castrates, and after 15 months they had increased further in weight. The muscular dystrophy and weakness were clearly more pronounced at the latter age, therefore it seems that this rapid increase in weight might possibly be attributed to the alarm reaction which has been described by Selye (12, 13).

Hatai (14), Anderson and Kennedy (15) and Winter and Emery (16) have all reported that gonadectomy induced hypertrophy of the adrenal in male rats, but atrophy in the female. The results of the present report confirm these observations. (See also table 2.)

The width of the cortex and medulla was measured from histological sections with an ocular micrometer to determine which part of the gland was responsible for the weight increase. In the castrate and E-deficient animals, the width of the fasiculate and reticular layers of the cortex was increased, the greatest increase occurring in the castrates. The glomerular layer was unchanged in width. Both groups showed the greatest increase over normals at 13 months of age. The data of Drummond et al. (2) do not show any increase in adrenal weight of male E-deficient rats, whereas those of Evans et al. (6) show a definite increase. The present data agree with those of Evans and co-workers.

4. Involution of the thymus did not occur as early in the E-deficient and castrate male rats as in the normals. Involution in the latter had taken place at 7 months of age, but it did not occur in the E-deficient animals until 13 months of age. It is possible that the thymus involuted earlier than 13 months, but data are not available because animals were not autopsied in the interval between 7 and 13 months.

Since it is generally accepted that androgen and estrogen hasten the involution of the thymus, the results of the E-deficient animals are difficult to explain. Apparently more male hormone was being produced in these animals, as was evidenced by the increase in the accessory gland weights, but regardless of this increase, thymus involution did not occur as soon as in the normal animals.

5. The thyroids of the E-deficient males were significantly heavier than the thyroids of normals and castrates at all ages. The greatest enlargement occurred in the 6 month E-deficient animals. Supplementing the diet with wheat germ oil gave thyroids that were only slightly heavier than those of normals. The addition of iodine to the E-deficient diet main-

tained the thyroid weight at the normal level and also maintained normal thyroid histology. These data will be presented in a subsequent report.

6. Vitamin E-deficiency in the male produced an increase, which was statistically significant, in the weight of the pituitary gland of the groups sacrificed at 4 and 7 months of age, but there was not a significant increase in the group sacrificed at 13 months. The percentage increase was 21, 23 and 3 per cent at 4, 7 and 13 months respectively. These data indicate that there was an influence on pituitary weight after 3 and 6 months but not after 12 months of E-deficiency. The pituitary glands of animals that received a supplement of wheat germ oil were 24 per cent heavier than those of normals. This increase, which is significant statistically, is approximately the same as that of E-deficient animals of the same age. The germinal epithelium was normal in these animals regardless of the increase in size of the pituitary gland. The reason for this weight increase is not known. The data of Evans et al. (6), from a much smaller number of animals, likewise show an increase in the weight of the pituitary gland of E-deficient and E-deficient animals given a supplement of wheat germ oil. However, the increase was considered non-significant by these workers.

Castration significantly increased the weight of the pituitary gland over that of normal animals at each age, the percentage increase being 78, 102, and 72 per cent at 4, 7, and 13 months respectively.

Endocrine gland weights of female rats. The autopsy data of the 170 female rats reported in table 2 are summarized as follows:

1. Vitamin E-deficiency did not significantly influence the weight of the ovary in any of the age groups studied. The cyclic function of the ovary was maintained for at least 12 months of E-deficiency, for it was found that females at this age had normal estrous cycles. Evans et al. (17) have previously reported that the estrous cycles of E-deficient females were normal.

2. The adrenal weight of the 6 month ovariectomized females was significantly less than the adrenal weight of normal and E-deficient animals of the same age. Hatai (14), Anderson and Kennedy (15) and Winter and Emery (16) have reported the same observation in ovariectomized female rats.

The difference between the adrenal weights of the E-deficient and normal animals was not significant at any age. Blumenfeld (18) reported that vitamin E-deficiency produced an atrophy of the medulla but a hypertrophy of the cortex in female rats. However, the absolute weight of the gland was not significantly different from that of normals. In the present study there was no alteration in absolute weight of the adrenal or in the width of the cortex or medulla.

3. The difference between the thymus weights of the E-deficient and normal females was not significant at any age at which comparisons are possible. The difference between the thymus weight of the normal and ovariectomized animals is significant, which indicates that ovariectomy delays thymus involution.

4. The thyroid hypertrophy obtained in the male E-deficient animals was not evidenced in the females, for there was no significant difference in thyroid weights of any of the groups.

TABLE 2

Autopsy data of normal, vitamin E-deficient and ovariectomized female rats

TREATMENT	AGE AT AUTOPSY	NO. RATS	BODY WT.*	GONADS	ADRENALS	THYMUS	THYROID	PITUITARY
	mo.							
E-def. 3 mo.	4	34	223 ±3.48	47 ±1.83	49 ±1.20	306 ±10.48	14 ±0.30	9.9 ±0.22
Normal	7	33	237 ±3.28	48 ±1.34	55 ±1.19	199 ±9.75	17 ±0.69	12.4 ±0.36
E-def. 6 mo.	7	36	230 ±4.59	43 ±1.62	58 ±1.54	222 ±8.36	15 ±0.42	12.7 ±0.36
Ovariectomized 6 mo.	7	31	260 ±4.47		45 ±1.57	234 ±7.68	15 ±1.10	12.3 ±0.28
Normal	13	6	245 ±6.48	33 ±3.09	66 ±5.10	138 ±7.62	15 ±1.08	13.9 ±1.13
E-def. 12 mo.	13	30	221 ±3.90	38 ±2.12	68 ±1.67	168 ±9.20	17 ±0.42	14.8 ±0.40

* The body weight is in grams; all other weights are in milligrams. The standard error of the mean of each weight is also given.

5. Vitamin E-deficiency did not alter the weight of the pituitary gland from that of normal female rats. This has been reported previously by Nelson (1) and Stein (19). Ovariectomy likewise did not influence the weight of the pituitary gland. This is in agreement with the reports of the majority of investigators, which have been summarized by Lawson et al. (20).

Assay of pituitary glands for gonadotropic hormone. The results of the pituitary assays of the various groups of rats are presented in tables 3 and 4. The average adrenal and thyroid weights of the hypophysectomized test animals indicate that in general the larger quantities of pituitary powder gave heavier adrenals and thyroids than the smaller

TABLE 3

Assay of male rat pituitary glands

DONOR				HYPOPHYSECT. RECIPIENT*			
Treatment	Age	Time stored	Amount injected	Av. ovar. wt.**	No. rats with corpora lutea	Av. adren. wt.**	Av. thyr. wt.**
	mo.	*mo.*	*mgm.*	*mgm.*			
Normal	4	7	1	10	0	8.7	5.0
			2	56	0	8.0	4.5
			4	69	1	7.5	5.8
			6	65	0	9.0	3.9
E-def. 3 mo	4	6	1	14	0	9.5	6.7
			2	46	1	8.6	4.5
			4	76	2	9.1	4.5
			6	94	3	9.0	4.8
Castrate 3 mo	4	5	1	14	1	7.5	5.7
			2	90	3	7.5	3.3
			4	78	3	8.9	4.6
			6	133	3	8.3	4.7
Normal	7	11	1	5	0	6.1	3.1
			2	20	0	8.2	5.9
			4	35	1	8.1	5.9
			6	53	0	8.0	5.8
E-def. 6 mo	7	15	1	7	0	7.4	3.9
			4	49	0	7.3	5.5
			6	47	3	8.8	5.8
E-def. + wht. germ oil 6 mo	7	6	1	8	0	8.3	4.0
			2	19	0	8.1	5.0
			4	41	0	7.6	5.4
			6	46	0	9.0	6.0
Castrate 6 mo	7	8	1	27	1	6.3	3.9
			2	76	3	8.3	4.9
			4	90	3	8.2	4.8
			6	83	3	9.0	6.0
Normal	13	8	1	7	0	6.8	3.9
			2	22	0	7.4	4.2
			4	49	0	8.3	5.3
			6	65	2	9.0	5.0
E-def. 12 mo	13	8	1	8	0	6.8	4.2
			2	21	0	6.8	4.5
			4	49	1	7.4	4.9
			6	60	3	7.7	4.3
Castrate 12 mo	13	1	1	22	1	7.7	4.7
			2	69	3	8.0	3.8
			4	81	3	8.0	5.0
			6	78	3	8.8	4.9

* The average ovarian, adrenal and thyroid weight of 5 operated controls was 4, 7, and 3.7 mgm. respectively.

** Average of 3 animals.

quantities. There is no indication that the thyrotropic or adrenotropic hormone content of the E-deficient pituitaries was increased. From the increase in thyroid weight, especially of the 6 month E-deficient males, it might be expected that more thyrotropic hormone was produced, but the data do not show any increase. If a test animal were used which was more sensitive to thyrotropic hormone, an increase might be detected.

TABLE 4

Assay of female rat pituitary glands

DONOR				HYPOPHYSECT. RECIPIENT*			
Treatment	Age	Time stored	Amount injected	Av. ovar. wt.**	No. rats with corpora lutea	Av. adren. wt.**	Av. thyr. wt.**
	mo.	*mo.*	*mgm.*	*mgm.*			
E-def. 3 mo.	4	6	1	7	0	9.0	3.0
			4	9	0	7.8	4.0
			6	11	0	8.0	5.0
Normal	7	7	1	5	0	7.3	3.6
			4	7	0	7.5	4.6
			6	7	0	8.7	4.7
E-def. 6 mo.	7	3	1	5	0	7.6	3.6
			4	7	0	7.6	4.9
			6	8	0	8.0	4.4
Ovariectomized 6 mo.	7	5	1	16	0	6.7	3.9
			4	69	3	7.8	4.7
			6	93	3	9.0	6.0
E-def. 12 mo.	13	½	1	7	0	7.5	5.0
			4	6	0	8.3	4.2
			6	6	0	7.6	4.6

* The average ovarian, adrenal and thyroid weight of 5 operated controls was 4, 7 and 3.7 mgm. respectively.

** Average of 3 animals.

The ovarian weights obtained from the assay of male pituitary glands were analyzed statistically by the technique of analysis of variance (21) to determine whether the apparent differences between ovarian weights were significant. This type of analysis allows the probabilities of chance differences of the size found between normal and E-deficient or normal and castrate animals for each dosage or age, to be combined into a single probability for testing the significance of the average difference over all groups.

The data in table 5 show that there are no highly significant[4] differences between the response from any particular dosage of E-deficient pituitary over all ages and the response from the same dosage of normal pituitary. The difference at 1 mgm., though on the border-line of significance statistically, is not considered significant biologically because of the small amount of stimulation above the control level of non-injected hypophysectomized controls. On the other hand, all of the differences between the response from any particular dosage of castrate pituitary over all ages, and the response from the same dosage of normal pituitary, are significant with the exception of the difference at 4 months. The 4 month castrate group, however, was consistently higher than the normal group

TABLE 5

Probability of the differences between ovarian weights occurring by chance in the assay of male rat pituitary glands

PROBABILITY COMBINATIONS	GROUPS COMPARED	
	Normal and E-deficient	Normal and castrate
	Probability	*Probability*
1. All ages at each dosage (i.e., 1 mgm. at 4, 7, 13 mo. etc.):		
1 mgm.	0.04	0.002
2 mgm.	0.37	0.0002
4 mgm.	0.33	0.03
6 mgm.	0.49	0.01
2. All dosages at each age (i.e., 1, 2, 4, 6 mgm. at 4 mo. etc.):		
4 mo.	0.51	0.30
7 mo.	0.27	0.004
13 mo.	0.60	0.002
3. All ages at all dosages (i.e., 1, 2, 4, 6 mgm. at 4, 7, 13 mo.)	0.26	<0.001

at all dosages, thereby agreeing with all other comparisons in the direction of the difference between castrate and normal.

The foregoing analysis shows that there is no significant difference in the ovarian weights obtained from the assay of normal and E-deficient male pituitary glands on a per unit weight basis, regardless of comparisons of the different combinations that were made. If, however, our assays were performed on a whole gland basis it would seem that the potency of the male glands would probably be increased after 3 and 6 months of E-deficiency. The percentage increase in total weight of the pituitary gland at these ages was 21 and 23 per cent, so if 21 to 23 per cent more pituitary powder were injected, it is probable that there would be an increase in the ovarian weights obtained. Whether such an increase would

[4] A probability >0.05 was considered to be statistically nonsignificant, 0.01 to 0.05 significant, and <0.01 highly significant or indicative of a real difference.

be statistically significant cannot be predicted. No increase in ovarian weight would be expected after 12 months of E-deficiency because there was no increase in the total weight of the pituitary gland at this age. Therefore, upon a per unit weight basis of pituitary material the present results do not confirm those of Nelson (1) and Drummond (2), but if the assays were performed on a per gland basis, it is probable that they would after 3 and 6 months of E-deficiency, but not after 12 months.

The assays of the female pituitary glands (table 4) do not show any difference in the ovarian response of the E-deficient and normal glands. The low level of response from the highest dosage of pituitary makes conclusions regarding the relative potency impossible. The pituitaries from the females that were ovariectomized for 6 months gave a substantial increase in ovarian weight, therefore it may be concluded that these glands are more potent than either the E-deficient or normal pituitaries.

To ascertain whether there was any deterioration of pituitary powder prepared and stored in the manner described above, a pooled sample of dried rat pituitary powder was assayed on normal 21 day-old female rats at intervals during a period of 16 months. Each rat in each assay received 2 mgm. of the dry powder. The average ovarian response obtained after storage for 4 months was 64 mgm. (3 rats); after 7 months, 64 mgm. (7 rats); and after 16 months, 58 mgm. (6 rats). These results indicate that there was no appreciable loss in potency when dried pituitary powder was stored for as long as 16 months. In view of these data it seems unlikely that there was any loss in potency of the pituitary powder from the normal, E-deficient and castrate rats reported above, for the longest period of storage of pituitary powder from these groups was 15 months.

The number of rats in each group having corpora lutea in their ovaries following the injection of the pituitary powder was taken as the criterion of the amount of luteinizing hormone in the pituitary material. The data in tables 3 and 4 indicate that castrate pituitary glands contain more luteinizing hormone (LH) than E-deficient glands, and that E-deficient male pituitaries contain more than those of normals. The pituitary glands from ovariectomized females were the only ones of this sex that formed corpora lutea in the ovaries of the test animals. Emanuel (22), Evans et al. (23), and more recently Hellbaum and Greep (24) have reported that luteinizing hormone is increased in amount in the pituitary gland of castrate animals.

The increased luteinizing capacity of the pituitary glands of the E-deficient males is correlated with the following anatomical alterations in these animals: 1, degeneration of the germinal epithelium; 2, increase in size of the accessories after 3 and 6 months, but no increase after 12 months of E-deficiency; 3, increase in weight of the pituitary gland after

3 and 6 months, but no increase after 12 months of E-deficiency, and 4, increase in size and number of basophils in the anterior lobe of the pituitary gland after 6 months of E-deficiency. There is no indication from the data that the follicle stimulating hormone (FSH) content of these pituitary glands was also increased although it may have been. However, the increase in size of the accessory glands of the 3 and 6 month E-deficient animals and the increase in amount of luteinization obtained in the assay of their pituitary glands, are definite indications that the LH content of the pituitary gland was increased. Although there was some evidence of an increase in luteinizing activity of the 12 month E-deficient male pituitaries, there was no increase in the weight of their accessory glands when compared with control animals.

When the data from the assay of the castrate male pituitary glands were plotted, it was found that the ovarian weights obtained as a result of injecting the pituitaries from the 3 months castrates, did not reach a plateau on the highest dosage of pituitary powder (6 mgm.). The curves from the assay of the 6 and 12 month castrate pituitaries were practically identical, for each attained a maximum at 4 mgm. of pituitary powder. There was no further increase in the height of the curve with 6 mgm. of pituitary material, but rather a slight decrease. This difference in response between the 3 month castrates on the one hand and the 6 and 12 month castrates on the other, indicates either that the pituitary glands of young adult castrate rats are more potent than those of older animals, or that the rate of increase in activity following castration is most rapid at a young age, after which a decline in potency occurs. Whether the increase in potency of the castrate pituitary glands represents an increase in FSH as well as LH (for it is probable that more luteinization and hence a greater ovarian weight would be obtained merely by increasing the quantity of LH), or whether both components increase simultaneously, is not indicated by the present data. Careful quantitative studies must be made with hypophysectomized female rats, using varying ratios of follicle stimulator to luteinizer, before this can be answered.

From the fact that the gonadotropic potency of the E-deficient pituitaries did not increase following the destruction of the germinal epithelium, the question arises as to the relative importance of the germinal epithelium and the interstitial tissue of the testes in the reciprocal gonad-pituitary interrelationship. It is evident from the data that removal of both the germinal epithelium and interstitial tissue by castration, greatly increased the gonad stimulating activity of the pituitary gland. Removal of the germinal epithelium alone by vitamin E-deficiency did not increase the gonadotropic activity; therefore it would seem that the interstitial tissue is more important in the control of the pituitary gland. Witschi et al. (25) have reported that there was more gonadotropic hormone

in the blood stream of x-ray sterilized males in parabiosis with normal females, than in normal animals. However, no attempt was made to assay the pituitary glands of the parabiotics, so their data cannot be directly compared with those presented in this report because they determined the amount of gonadotropic hormone in the bloodstream and not in the pituitary gland.

We are greatly indebted to Dr. A. B. Chapman of the Department of Genetics for assistance in the statistical analysis of the data obtained from the assay of the pituitary glands.

SUMMARY

1. Endocrine gland weights of 234 vitamin E-deficient male and female rats, and 247 gonadectomized and normal male and female rats which served as controls for the E-deficient animals, are presented. An analysis of the data shows the following effects of vitamin E-deficiency:

a. Degeneration of the germinal epithelium with a consequent decrease in testis weight to a level which was less than one-half that of the testes of normal animals. There was no effect on the weight or on the cyclic function of the ovary.

b. An increase in accessory gland weight after a short period of deficiency (3 mos.), but a decrease after longer periods (15 mos.). Supplementing the diet with wheat germ oil produced the heaviest accessory glands obtained in any of the animals.

c. The adrenals of the males increased in weight due to hypertrophy of the cortex, whereas there was no influence on the weight of the adrenals of the female.

d. Involution of the thymus was delayed in the male but not in the female.

e. The thyroids of the males increased as much as 100 per cent in weight, whereas those of the females were not affected. The increase in weight of the male thyroids was largely prevented by supplementing the diet with wheat germ oil, and completely prevented by supplementing with iodine.

f. The pituitaries of the males increased in weight after 3 and 6 months of E-deficiency. There was no increase after 12 months of deficiency. The basophils increased in size and number after 6 months of deficiency. The weight of the female E-deficient pituitary gland was not different from that of normals.

2. Statistical analysis of the ovarian weights obtained following the injection of E-deficient male and normal male pituitary powder into immature hypophysectomized female rats, failed to show a significant difference in the gonadotropic potency. Pituitary glands from castrate rats

produced a significant increase in ovarian weight over that produced by normal glands. Pituitaries from E-deficient females produced the same ovarian response as those from normal females.

3. There was an increase in the luteinizing hormone content of the pituitary glands of vitamin E-deficient males when compared with normals. This increase was not as great as that obtained with those of castrate males.

4. Data are presented which show that there is no appreciable loss in potency of dried rat pituitary glands stored for as long as 16 months.

5. The bearing of the results upon the general problem of the gonadal-pituitary interrelationship is discussed.

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A CORRELATION OF THE pH OF ARTERIAL BLOOD AND URINE AS AFFECTED BY CHANGES IN PULMONARY VENTILATION[1, 2, 3]

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This investigation was undertaken primarily to study simultaneously the pH changes in arterial blood and urine as measured continuously with glass electrodes and produced by variations in composition of the respiratory gases. Urine analyses were made for ammonia, phosphates and chlorides for information relative to the concentration of substances concerned in the urine pH changes.

METHODS. Ten to fifteen kilogram dogs anesthetized with sodium pentobarbital were used in these experiments. Ureteral catheters (French no. 4 or no. 5) were shortened and used as cannulae for the ureters exposed by a retro-peritoneal incision. A glass electrode of the condenser type having a capacity of 0.2 to 0.5 cc. was attached to the left ureter. For arterial pH a bulb type of electrode fitted into a cannula (Fruhling and Winterstein, 1933) was mounted in the left carotid artery of heparinized animals. Continuous records of both urine and blood pH were made on smoked paper as previously described (Brassfield, 1936).

To insure a flow of urine sufficient for these studies a constant intravenous injection of isotonic saline was maintained by a small injection pump. A Gibbs drop recorder delivering 25 drops per cubic centimeter was used to record the flow of urine from the right ureter. Mean blood pressure and pulmonary ventilation were recorded in the usual manner.

The urine after having passed through the glass electrode was collected under toluol in 1 cc. samples and immediately placed in a small icebox until the analyses could be made. The samples were analyzed for ammonia nitrogen by Conway's micro-ammonia method (1933), inorganic phosphate by Kuttner and Lichtenstein's modification (1930) of the Bell-Doisey method, and chloride by Sendroy's micro-chloride method (1937).

RESULTS. Preliminary experiments were performed to ascertain the

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[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan by Vivian G. Behrmann.

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reliability of the continuous pH method. In one series of experiments a glass electrode was attached to the cannulated ureter for recording continuously the pH values. The urine flowing through the electrode was collected for pH determinations on a set adapted for single samples at 37°C. and the results plotted on the same record with the continuous pH curve. A similar series was obtained with arterial blood. Blood samples from the left femoral artery served to check the continuous blood pH curves. Figures 2 and 1 are illustrative of these experiments. The close

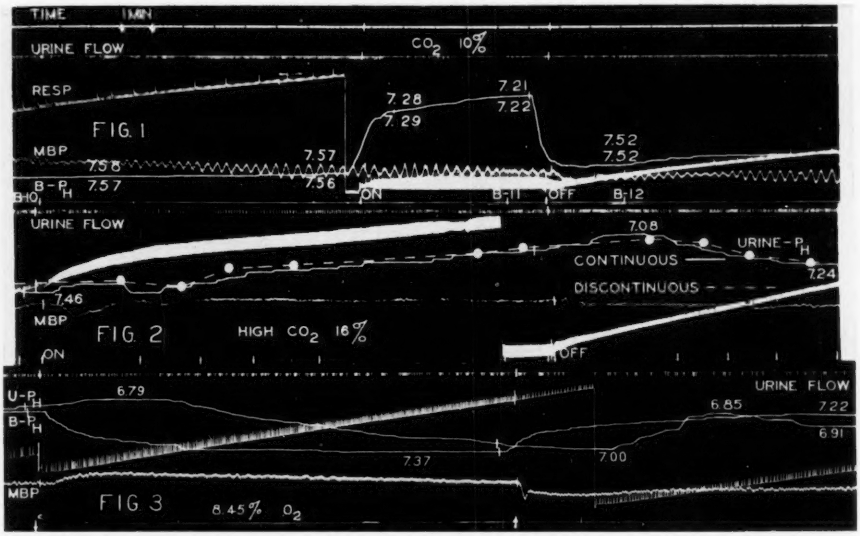


Fig. 1. Effect of 10 per cent CO_2 on arterial blood pH. Numbers above the continuous blood pH curve (B-P_H) were obtained from the calibration of the continuous electrode; the numbers below the curve were obtained from samples taken and the pH determined on another electrode.

Fig. 2. Comparison of continuous and discontinuous urinary pH curves during and after the administration of 16 per cent CO_2.

Fig. 3. Record showing continuous pH curves for both urine and blood in a low oxygen experiment.

agreement between the continuous and discontinuous records indicates that the continuous method used here is a reliable indicator of the pH values.

Carbon dioxide mixtures. Intra-tracheal administrations of carbon dioxide, oxygen, and nitrogen mixtures in which the carbon dioxide varied from 7 to 16 per cent, were made. Results from 21 out of 26 procedures showed that a sudden decrease in blood pH was followed by a gradual decrease in urinary pH, which culminated at the end of the procedure

in a marked decrease of short duration followed by an immediate increase on return to room air. This marked decrease, occurring at the close of the procedure, was usually more prominent in cases where the urine flow was either very sluggish or stopped during the carbon dioxide administration, but increased in rate at the end of the procedure. Thus, the acid products accumulated during the carbon dioxide excess were not removed until urine flow was initiated on return to room air. In five instances, a slightly increased pH occurred in the urine just after the onset of the procedure, but previous to the gradual decrease in pH. Figure 2 is illustrative of these results. This record shows an administration of 16 per cent carbon dioxide for 17 minutes. The urine pH changed from 7.46 before the carbon dioxide administration to 7.08 two minutes after the close of the administration; then increased to a pH of 7.24 six minutes later. The blood change, which is not depicted here, but determined from arterial samples, was from a pH of 7.41 previous to the administration to a pH of 7.08 during the procedure. On return to room air, the blood pH increased to 7.38 within 60 seconds. Figure 1 is demonstrative of a continuous blood pH curve obtained by subjecting the dog to a 10 per cent carbon dioxide mixture. The sudden acid change, brought about in the blood, is shown by the sharp rise in the blood pH curve, which falls on return to room air to a pH 0.05 or 0.06 less than the pre-administration pH. In some cases a slight over-shooting has been observed, that is, the blood has attained a greater pH immediately following the administration, with a return to the pre-administration pH in a few minutes. Gesell and Hertzman (1926) noted this when following blood changes with the manganese dioxide electrode. The urine samples collected during the carbon dioxide administration recorded in figure 1 showed a pH decrease of 0.38 pH.

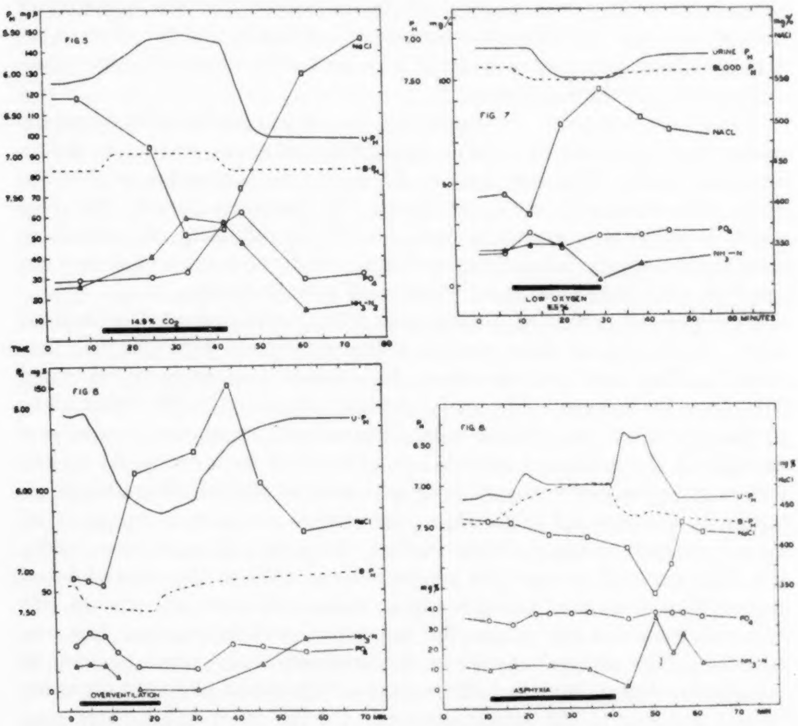
The blood pH changes observed varied between 0.12 and 0.38 pH, depending on the amount of carbon dioxide in the gas mixture. The urine pH changes were of a much greater magnitude, ranging from a pH change of 0.20 to one of 1.02.

Administration of carbon dioxide definitely slowed urinary secretion. In the 16 per cent CO_2 procedure (fig. 1) the rate was slowed from 28 drops per minute to 16.1 drops per minute. Return to room air increased the flow to 25.8 drops per minute. In some cases where the pre-administration urinary secretion was small the carbon dioxide produced anuria, as shown in figure 1, although the CO_2 concentration was only 10 per cent. These two procedures are illustrative of the results obtained on the rate of urine flow under carbon dioxide.

The latent period for the effects of CO_2 administration to alter the blood pH was as short as 5 seconds, whereas the urine pH curve required at least 50 seconds after the blood change appeared. In cases characterized by a very slow urinary output or a complete anuria, the change in the

urine curve did not appear until the flow was initiated or until the rate increased. Thus due to the capacity of the space between the electrode and epithelium of the kidney the latent period of the urinary pH change as recorded is much too long.

The micro-analyses made on the urine showed a chloride decrease and an ammonia and phosphate increase coincident with the decrease in urinary pH. Data plotted in figure 5 are typical of the results obtained during 3



Figs. 5, 6, 7 and 8. Curves for urinary and arterial blood pH and urinary NaCl, PO_4 and NH_3-N during changes of pulmonary gases and ventilation.

carbon dioxide administrations. It is to be noted that the least chloride excretion, and the greatest ammonia and phosphate excretion coincide with the lowest urinary pH. These findings are in keeping with numerous reports on acid, ammonia, chloride and phosphate excretion with the exception of the data of Havard and Reay (1926), who noted no ammonia formation on administration of 6 to 8 per cent CO_2 to humans, nor did they observe any significant change in chloride excretion.

In reported experiments performed on man, diuresis usually resulted as a consequence of the administration of carbon dioxide. The anuria or decrease in urine formation which occurred in these experiments is in agreement with the results of Adolph (1935) on frogs. The actual mechanism of the depressed function in the anesthetized animal is as yet undetermined. The anesthesia may be a factor in bringing about the anuria. Considering the hypothesis of Adolph that local pressure changes may occur in the kidney as a result of carbon dioxide intoxication, being either a constriction of the afferent arterioles or a relaxation of the efferent arterioles or both, one may postulate that a similar circulatory change occurs in the region of the dog kidney.

Mechanical asphyxia. Mechanical asphyxia was produced by clamping with a large hemostat the rubber tubing connecting the trachea to the rebreathing tank. This was done at the end of an inspiration so that the lungs were filled with room air during the asphyxial period. In three cases, a progressive asphyxia was obtained by allowing the animal to breathe through the rebreathing system in which the volume of air was cut down to a few liters and the CO_2 allowed to accumulate.

Twenty-eight asphyxial experiments were performed on anesthetized dogs. In 8 of these there was no urinary secretion following the procedures. The length of time that the animals were asphyxiated varied from 40 to 90 seconds. The blood showed a decrease in pH within 10 to 20 seconds after the trachea was clamped and continued to decrease throughout the asphyxial period. At the end of each asphyxial period, a marked hyperpnea brought about an increased pH which gradually returned to the pre-asphyxial value. Immediate anuria was typical of all cases. As soon as the secretion started, the urine pH curve rose rapidly and then dropped toward the previous level. While the arterial blood always showed an acid increase within 10 seconds after the trachea was clamped, the urine pH change did not occur until the urinary flow was initiated. The marked change in the gradient of the curve as soon as urinary flow began, may be indicative of a diffusion of CO_2 into the urine formed previous to the administration. On the other hand, there may have been a greater reabsorption of basic substances in the tubules, to alleviate the state of acid excess. Progressive asphyxia resulted in a gradual decrease in both blood and urine pH.

Urinalysis for chlorides, ammonia and phosphate of the samples obtained immediately following the asphyxial period gave evidence of a decreased chloride excretion, an increased ammonia formation, but no appreciable change in phosphate elimination. Five out of 6 asphyxia experiments caused a decrease in chloride excretion, while one showed no apparent change. Ammonia formation increased in amounts ranging from 10 to 38 mgm. per cent. Phosphates did not change appreciably in

5 instances, although one case brought forth data showing an increase of 40 mgm. per cent after the asphyxial period was over.

In figure 8 are shown curves typical of progressive asphyxia. While the blood pH shows a gradual decrease throughout the entire period and reaches its lowest value at the end of the procedure, the urine pH, chloride and ammonia show the greatest change 8 minutes following the greatest blood change. As in CO_2 administration the lowest chloride value and the highest ammonia value coincide with the lowest urinary pH, which may be indicative of the hydrogen ion acting as a common stimulus in the two cases. The anuria could be brought about as a result of carbon dioxide and low oxygen acting separately or together upon the mechanisms controlling the flow of blood through the kidney.

Low oxygen. Intra-tracheal administrations of low oxygen mixtures (6–10 per cent) were made for periods of time ranging from 10 to 35 minutes. In 21 procedures the low oxygen produced an alkaline change in the blood, which shifted back to normal immediately on return to room air. The urine increased in pH also, but it was a gradual change. Although the magnitude of the urine pH change was usually more than twice that of the blood pH change, the gradual slope of the urine curve did not reach its limit until shortly after the procedure was ended. In 4 cases the urine showed an acid change previous to the alkaline trend, whereas 3 instances produced no significant alteration in the urine pH.

A 16½ minute administration of 8.45 per cent oxygen (fig. 3) caused the typical results of a rise in blood pressure, a hyperpnea and a diminution in the urinary flow. The rate decreased from 14 drops/minute to 10 drops/minute during the administration, with a rate of only 6 drops/minute on return to room air. Results were obtained in which the recovery, as regards rate of flow, was both incomplete and complete. In this administration of low oxygen the blood increased 0.15 pH and showed an exact reversal on return to room air. The urine pH increased from 6.79 four minutes after the onset of the administration to 7.00 three minutes after the return to room air, whereupon it decreased to 6.85 within the next four minutes.

It was noted that the lower the oxygen percentage, the greater were the changes in the blood and urine pH. The effect of the anoxia on the blood pH was usually seen within 10 seconds after the onset of the procedure. The urine, however, usually required 2 to 5 minutes before the alkaline trend occurred. The rate of flow was undoubtedly a factor in preventing the urine pH change from appearing on the record as soon as it occurred in the kidney.

Urinalysis was made on 6 low oxygen procedures. From the data obtained it appeared that the phosphates showed no significant change in 5 procedures. One period of anoxia decreased the phosphate elimination.

Ammonia formation fell in 5 out of 6 administrations while one low oxygen procedure caused no change in the rate of ammonia formation. The chloride excretion rose in 3 out of 3 cases. A graphic representation of results in a 9.5 per cent low oxygen administration for 20 minutes and 50 seconds as shown in figure 7 demonstrates a blood pH increase of 0.14 and a urine pH increase of 0.33 pH. Both curves reversed on return to room air. Urine formation in this procedure decreased from 11.5 drops/minute to 7.0 drops/minute during the anoxia. Recovery increased the rate to 12 drops/minute. The urinary constituents showed the typical directional changes of low oxygen. The lowest ammonia and the highest chloride values were determined from the sample which corresponded to the most alkaline pH. These findings on urinary constituents are similar to those found in humans by many investigators.

It appears that the results, which Toth (1937) obtained in his study of anoxia in dogs, showed a parallelism between chloride and water excretion. In the anesthetized sacrifice dogs, an oliguria developed, with an accompanying low chloride excretion. Unanesthetized bladder-fistula dogs responded to low oxygen with a polyuria and a coincident rise in urinary chloride. Toth suggests that anoxemia may result in an asphyxia of the tubular epithelium, with a consequent interference with the process of reabsorption. This would entail a polyuria approaching plasma composition. His experiments, however, did not show a urine of plasma composition on initiation of polyuria. The low urinary rates in our experiments, accompanying the high chloride excretion, indicate a lack of correlation between water and chloride excretion in anoxemia on the anesthetized dog.

Overventilation. Overventilation was produced by an electrically driven pump in closed circuit with the rebreathing tanks. Fifteen procedures of varying duration (5–30 min.) were performed. The blood always showed an alkaline trend varying from 0.18 to 0.32 pH depending upon the magnitude of the ventilation. The majority of the procedures caused an immediate increase in the urine pH, accompanied by a diuresis. In 4 cases, the urine offered varied changes during the procedure, but has consistently shown a marked alkaline change after the overventilation. A decreased urinary flow may have been a factor in the recording of these results.

A graphic representation of a hyperventilation procedure (fig. 6) which induced an increase in urine formation, demonstrates the typical results. The rate of flow in this 20 minute procedure increased from 11 drops/minute to 15 drops/minute. At the close of the procedure the rate fell to 5 drops/minute. The blood pH began to increase from 7.23 to 7.50 within 10 seconds after the hyperventilation was begun while the urine had a latent period of 1 minute and 50 seconds before the pH began to increase

from 5.08 to 6.28. Recovery shows that both curves returned to approximately the same pre-administration level.

Characteristic changes in the urinary constituents are plotted on the chart. In each of 6 procedures, the chlorides showed an increase during the overventilation, although the degree of the rise varied with the duration and the degree of the hyperpnea. Ammonia formation and phosphate elimination decreased in 4 cases, while 2 procedures elicited no appreciable change. In the 2 cases in which the ammonia and phosphate were unaffected by the overventilation, the urinary pH changes were less prominent. Usually the most alkaline part of the urinary curve occurred simultaneously with the depression in ammonia and phosphate excretion and the increase in chloride elimination.

If one compares the charts, typical of low oxygen (fig. 7) and overventilation (fig. 6) it is easily seen that overventilation creates in all respects except urinary flow a similar picture to low oxygen effects. The total hyperventilation (56.7 liters/kgm./hr.) caused a blood pH change of 0.27 pH while the total ventilation induced by low oxygen (33.9 liters/kgm./hr.) changed the blood 0.14 pH. The urine of the hyperventilated animal increased 1.20 pH while that of the dog subjected to anoxia increased 0.33 pH. Phosphate elimination, though unaffected in the low oxygen procedure was found to decrease 19 mgm. per cent under overventilation. Ammonia formation fell to a level of 3 mgm. per cent under low oxygen and to zero under overventilation. The chloride rose 152 mgm. per cent under low oxygen and 103 mgm. per cent under the hyperventilation. Rate of urinary flow always decreased in the anoxial state but in the majority of the overventilation experiments it increased. The decreased rate of urine formation, characteristic of low oxygen is undoubtedly due to the low oxygen effects on the kidney. In the light of the work of Adolph (1936) and Toth (1940), these are purely of a circulatory nature. In overventilation, an abundance of oxygen prevents such an oliguria. In fact, a diuresis usually occurs.

SUMMARY AND CONCLUSIONS

Continuous blood and urine pH tracings under conditions produced by changes in pulmonary ventilation were recorded in the anesthetized dog. Results show a qualitative similarity between blood and urine pH, the extent of the change being smaller and of shorter duration in the blood than in the urine.

A study of the latent periods shows that the blood pH reacts to pulmonary ventilation changes within 5 to 20 seconds while the urine pH, requires a variable length of time after the blood change occurs. If the rate of flow through the electrode is rapid, the change may appear within

a minute; however, a slow flow or an oliguria lengthens the time before the urine electrode records the change.

Carbon dioxide and mechanical asphyxia caused a blood and urine pH decrease while low oxygen and overventilation produced an increase in blood and urine pH.

Ammonia formation, that mechanism of the kidney whereby base is conserved for the organism, was stimulated as a result of carbon dioxide and mechanical asphyxia. Low oxygen and overventilation produced an alkaline blood and suppressed the excretion of ammonia by the kidney.

Carbon dioxide administration caused an increase in the elimination of phosphates while that of mechanical asphyxia did not alter the urine phosphate materially. Overventilation caused a decrease in phosphate elimination while the alkaline state created by low oxygen was not sufficient to affect the amount of phosphate in the urine.

Chloride excretion was diminished under carbon dioxide and asphyxia, but increased markedly in the case of low oxygen and overventilation. Chloride diminution occurred in those cases where the phosphate elimination increased. This may be indicative of an inverse relationship between chloride and phosphate. Asphyxia, carbon dioxide and overventilation show chloride excretion changes, which are in the same direction as the rate of water excretion. In these cases, there may be a definite relationship between the chloride and the water excretion. Administrations of low oxygen show no correlation between water and chloride elimination.

Since the urine pH changes in the same direction as that of the blood and within such a short time after the blood changes together with the fact that the urinary constituents studied change in a direction such as to counteract the pH change of the blood seems to indicate that the hydrogen ion is the factor primarily concerned in these studies.

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FACTORS IN THE ABSORPTION OF INSULIN FROM THE ALIMENTARY TRACT[1]

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A number of reports from this laboratory (1, 2, 3) and others (4, 5) leave no doubt that insulin can be absorbed from the G. I. tract, but in no case has this absorption reached a practical amount (6), nor has a satisfactory explanation yet been given for the process of absorption of so large a molecule.

The object of this investigation was to find compounds and combinations of compounds that affect the absorption of insulin and to determine which, if any, of the well-known factors that in general influence absorption are involved in the case of insulin.

Effect of sodium amytal on the blood sugar of the dog. Since the sacrifice animals used were anesthetized with sodium amytal and the blood sugar was taken as an indication of the extent of the insulin absorbed, the effect of sodium amytal alone on blood sugar was studied. All of the animals used in these control experiments were subjected to operation and treated exactly as were the experimental animals with the exception that an isotonic solution of sodium chloride was placed in the isolated loop instead of a solution containing insulin. Blood glucose determinations were made by the method of Shaffer and Hartman as described by Shaffer and Somogyi (7), using the zinc precipitation procedure of Somogyi (8). Two dogs were given the amytal intravenously and intramuscularly in the ratio of 2:1. In three dogs the amytal was given intraperitoneally. The blood sugar changes of these five dogs are shown in figure 1.

In general there is a gradual falling off in blood sugar with time, amounting to an average of about 15 mgm. per cent in 12 hours. Therefore, to minimize corrections, the data on insulin absorption are divided into two groups: 1, the blood sugar changes when insulin was given within 5 hours after anesthesia, and 2, the changes after 5 hours following anesthesia.

Response of the amytalized dog to known quantities of insulin. In order

[1] Taken from a thesis submitted by R. L. D. in partial fulfillment of the requirements for the doctorate of philosophy, University of Rochester, June, 1940.

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to get some indication of the amount of insulin absorbed from the G-I tract, known quantities were given to the amytalized dogs subcutaneously and intravenously. The results given in figure 2 show that consideration must be made of the level of blood sugar at the time of administration; and in the experiments following, all data accumulated when the initial blood sugar was below 50 mgm. per cent were discarded.

Absorption of insulin in sacrifice dogs. (a) *Compounds and combination of compounds that have an effect.* The technique previously used (17, 3) of introducing insulin with test substances into a segment embracing the lower duodenum and upper jejunum of the anesthetized dog has been continued. The solutions used in this group of experiments uniformly had a pH of 4.5. The effect on blood sugar of the various solutions is

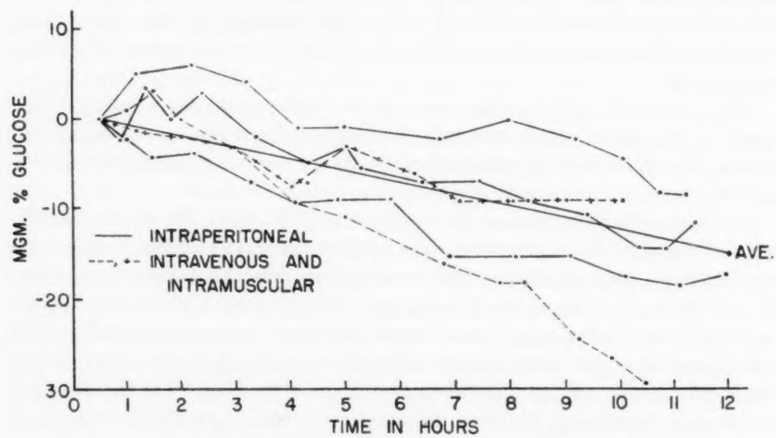

Fig. 1. Changes in blood sugar of dogs under amytal anesthesia

shown graphically in figure 3, where the upper bar with each entry indicates the average blood sugar fall induced by the solution when given within 5 hours after anesthesia, while the lower bar represents the fall 5 or more hours after anesthesia. One-half hour was allowed for absorption.

An examination of the data reveals that absorption falls off with time after anesthesia, and this factor must be considered in studying the data.

Comparing single compounds, we find the following order of effectiveness: pinacol (tetramethyl glycol) and methyl salicylate, thiamin, quinine and hexyl resorcinol, Aerosol O.T. (dioctyl sodium sulfosuccinate), and calgon (sodium hexametaphosphate). The reports of other workers on the effectiveness of pinacol (4), hexyl resorcinol (2) and quinine (Cutting, private communication) are confirmed, and methyl salicylate, thiamin, Aerosol O.T. and calgon are added to the list.

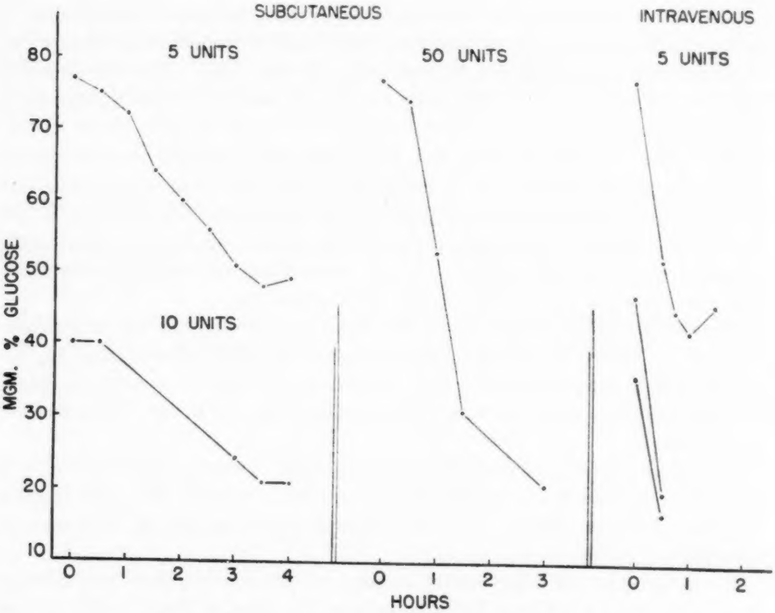

Fig. 2. Response of the amytalized dog to insulin

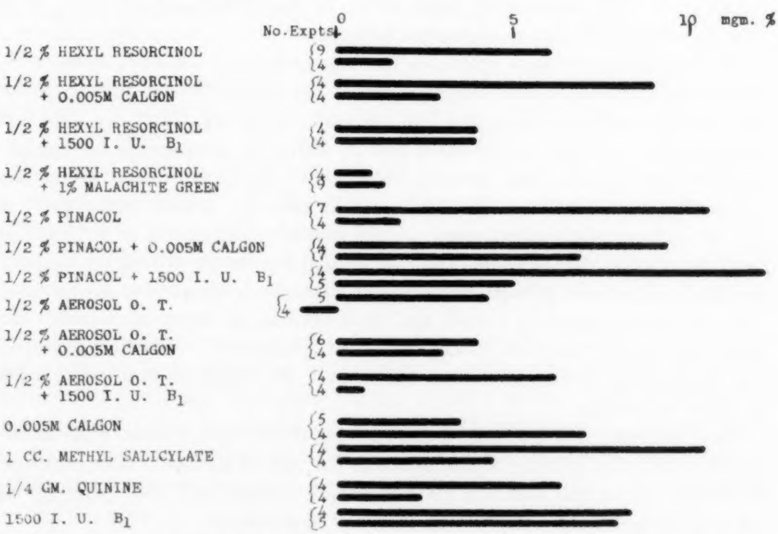

Fig. 3. Fall in blood sugar from 50 I. U. insulin in intestinal loop

The influence of methyl salicylate is in line with the observation of Lauter (private communication) that this reagent promotes the absorption of the alkaloids, morphine and strychnine, by the skin. The compound is quite irritating and its action may be due to an increased circulation in the intestinal mucosa.

The effect of thiamin may not have been due entirely to absorption, for Mosonyi and Aszódi (9) demonstrated that the intravenous injection of vitamin B_1 into normal men lowered the blood sugar 20 to 40 mgm. per cent and increased the insulin in the blood. However, in the experiments reported here, thiamin must have had some effect on absorption because when it was given alone the results were negative.

Calgon is a compound very powerful in its ability to tie up calcium ions (10). Since this ion has been reported to inhibit absorption (11, 12, 13, 14, 15), it was postulated that its removal would be conducive to an increased absorption of insulin. The assumption was borne out to a small extent only.

The good response to pinacol, which does not lower surface tension, and the poor response to Aerosol O.T., which does, confirm the observation (3) that surface tension, per se, cannot account for an increase in absorption.

A combination of compounds influencing absorption does not always show an additive effect. It is questionable whether one could always expect a summation, because it is not inconceivable that some of these compounds may react with each other to the detriment of both, even though no precipitates were seen at any time.

(b) *Substances without effect on the absorption of insulin.* Some substances that could conceivably promote the absorption of insulin from the gastro-intestinal tract and the reasons for trying them are listed in table 1. In the first group there are 28 different compounds having hydrophobic and hydrophilic groups, chosen with the view in mind of finding one with a structure such that it would fit into the insulin molecule by a sort of lock-and-key mechanism, in the manner postulated in the case of the bile salts and fatty acids (16). A logical procedure would be to hold, say, the hydrophilic group constant and vary the hydrophobic group until the best effect should be found, and then reverse the experiment until the most effective hydrophilic group should be discovered. But it was found that the two groups are interdependent, and one might as well follow a hit or miss procedure.

The importance of electrolyte ions in the absorption of other substances would seem to be of little consequence in the case of insulin. The addition of potassium to the solution gave negative results and the tying up of calcium with sodium citrate was likewise ineffective.

Since methyl salicylate, a mucous membrane-disturbing compound,

definitely promoted the absorption of insulin, other substances which irritate the intestine should behave similarly. However, this was not found to be true, and it appears that a mere change in the mucosa is insufficient: it must be one of specific character. Not even an increase in villi action will influence insulin absorption.

TABLE 1

Substances without effect on absorption of insulin grouped under reasons for trying them

Hydrotropic or lower surface tension	*Tie up calcium ion*
Tetramethyl ammonium hydroxide	Sodium citrate
dl-malic acid	
Diethyl acetic acid	*Have an irritating effect on or change character of the intestine*
Malonic acid	
Butyric acid	Saponin
Rochelle salt	Histamine phosphate
Lactic acid	Aspirin
Succinic acid	Strophantin
Calcium lactate	Yeast
Sodium cetyl sulfate	Ernutin
Aerosol M.A.	Digitonin
Aerosol O.S. sodium alkyl naphthalene sulfonic acid	
	Potassium aids absorption
Sodium glycocholate	Potassium chloride
Keto ethylresorcinol	
Keto propylresorcinol	*Increase villi action*
Keto butylresorcinol	Oil of clove
Keto pentyl resorcinol	Zinc sulfate
Butyl-b-resorcylate	Yeast
Butylsalicylate	
Butyl-p-hydroxybenzoate	
2,4-dihydroxybenzoic acid	
n-butyropyrogallol	
p-ter-butyl-phenol	
Tyrosine	
Salicylic acid	
Orcinol	
Thymol	
Amyl cresols	

Relation between surface tension and absorption. A large number of investigators have attached importance to surface tension as a factor in absorption, and the literature on the subject is too vast to attempt a review here. An examination of table 2 with compounds arranged in the order of decreasing effect on surface tension seems to dispose finally of the idea that this property is in some way closely related to the absorption of insulin (17).

Absorption of insulin in normal dogs. In these experiments the solution

containing insulin and the compounds to be tested were introduced into dogs' stomachs by a stomach tube. Samples of blood were taken before giving the solution and also one a half-hour and one an hour after administration. Eighty-seven experiments were done on eight normal dogs, about

TABLE 2

Effect of surface tension on absorption of insulin

SOLUTION	BLOOD SUGAR DROP	SURFACE TENSION
	mgm. %	*dynes/cm.*
½ per cent hexyl resorcinol	6	27
½ per cent Aerosol O.T.	4.3	29
½ per cent Aerosol M.A.	0	37
½ per cent Aerosol O.S.	0	39
Saturated sodium cetyl sulfate	0	45
½ per cent saponin	0	63
½ per cent sodium benzoate	0	66
½ per cent pinacol	10.4	66
0.005 molar butyl salicylate	0	68
0.005 molar calgon	3.4	68
Water	0	70
1500 I. U. B_1 (water solution)	8.2	72
0.005 molar butyl-b-resorcylate	0	73

TABLE 3

Compounds having no effect on the absorption of insulin when given by stomach tube

COMPOUND	NUMBER OF EXPERIMENTS
Trypan red	2
Urea	2
Saponin	4
*Vitamin B_1	4
*Aerosol O.T.	2
Benzoic acid	2
Lactic acid	2
Starch	2
Ferric hydroxide	2
Gum arabic	3
*Quinine	4
Sodium citrate	8
Malachite green	4
Oil of clove	2

* These compounds are effective in an isolated loop.

fifty of which were done on two dogs. The concentrations of the various substances used ranged from 0.1 to 1 per cent, the amount having little effect on the results. The substances tried under conditions which gave good responses with other substances, and therefore believed to have no effect in this type of experiment, are given in table 3.

In many cases the findings in the loop experiments were confirmed, while in others they were not. The mere presence of a substance which lowers surface tension has no effect, nor do the removal of calcium, increased villi action, or most intestinal irritants bring about an increased absorption. Malachite green and other substances supposed to inhibit enzyme activity (5) have no effect superimposed upon the effect of those

TABLE 4

Positive stomach tube experiments

	NO. OF EXPTS.	BLOOD SUGAR DROP	
		½ hr.	1 hr.
		mgm. per cent	*mgm. per cent*
Effect of pH:			
*pin + cal (pH 3.5)	1	+8	+6
pin + cal + buf (pH 10.1)	2	8	8
pin + hex + cal (pH 4)	6	6	2
pin + hex + cal + buf (pH 10)	9	12	4
Effect of serum:			
pin + hex + cal + buf	9	12	4
pin + hex + cal + buf + serum	2	29	24
Effect of hex:			
pin + cal + buf	2	8	8
pin + hex + cal + buf	9	12	4
Effect of pg:			
pin + hex + cal + buf	9	12	4
pin + hex + cal + buf + pg	2	31	15
Effect of ms:			
pin + hex + cal + buf	9	12	4
pin + hex + cal + buf + ms	3	22	20
Effect of composite:			
pin + hex + cal + buf + ms + pg	6	26	12

* hex = hexyl resorcinol; pin = pinacol; cal = calgon; buf = a sodium carbonate-bicarbonate buffer that will give the solution introduced a pH of about 10; pg = propylene glycol; ms = methyl salicylate.

compounds which give positive experiments. On the other hand, thiamin, Aerosol O.T. and quinine were found to be without action in the stomach tube experiments, but were definitely positive in the isolated loop. It must be remembered that these compounds were given in a solution containing other substances and a combination may have taken place rendering them useless.

The data in table 4 are presented for the experiments in which there are

no complicating outside influences other than the presence at times of one of the above-mentioned noneffective compounds.

It was thought best here to make preliminary experiments on a number of substances and try to build up an effective combination rather than spend too much time on a single questionable factor. Table 4 was developed following this procedure, which is justified by the progressive improvement shown. Clearly it is possible to build up a combination which summates the effects of different compounds. This summation was not always found, of course, as was also the case in the loop experiments.

Enzyme studies. These investigations were prompted by the observations of Daggs, Murlin and Murlin (18) that hexyl resorcinol protects insulin from hydrolysis by pepsin and of Young, Phillips and Murlin (17) that the compound inhibits the activity of trypsin. The effect of

TABLE 5

Enzyme studies

	PEPSIN ALBUMIN DIGESTED	TRYPSIN N/10 ALKALI	EREPSIN N/10 ALKALI
	mm.	*cc.*	*cc.*
Control	4.5	6.9, 7.2 > 7.1	3.7, 3.1 > 3.4
½ gram quinine	2.0	6.6, 5.8 > 6.2	3.7, 3.0 > 3.4
1 cc. 5 per cent pinacol	4.5	6.0, 6.6 > 6.3	3.8, 3.6 > 3.7
1 cc. 10 per cent calgon	0.0	6.9, 7.2 > 7.1	3.2, 3.3 > 3.3
0.1 gram hexyl resorcinol	0.0	4.6, 4.3 > 4.5	1.7, 1.8 > 1.8

some of the substances that promote the absorption of insulin on pepsin, trypsin and erepsin activity was investigated, and the results are compared in table 5. The procedures used were as follows:

Pepsin studies. Mett tubes were incubated at 37°C. in 30 cc. solution of 2½ per cent Bausch and Lomb flake pepsin in N/20 HCl for 30 hours. The amount of albumin digested was measured. The figures in table 5 are the average result of 4 determinations.

Trypsin studies. Twenty cubic centimeters of 1½ per cent fat-free casein plus 10 cc. of Baker and Adamson trypsin plus 0.5 cc. of toluene were incubated for 32 hours. A 10 cc. aliquot was taken to which was added 10 cc. of neutral formaldehyde and 100 cc. of water. The solution was titrated with N/10 alkali.

Erepsin studies. Ten cubic centimeters of 2½ per cent bacteriological peptone plus 20 cc. of 5 per cent Difco erepsin plus 0.5 cc. toluene were treated exactly as described above.

Quinine, a protoplasmic poison, was shown by Cutting (private communication) to promote the absorption of insulin in the intestine of rabbits,

and this observation was confirmed in the isolated loops of dogs. Although the compound inhibits pepsin activity, it proved ineffective in stomach-tube experiments, where this activity would be important, and was effective in intestinal loop experiments where it could not be important. Presumably its action in the dog is exactly the same as in the rabbit.

Pinacol has no appreciable effect on any of the enzymes while calgon inhibits the activity of pepsin. These facts do not furnish any explanation of the absorptive activity previously noted, of the substances named.

The most important observation is that hexyl resorcinol inhibits the activity of all three enzymes and furnishes insulin protection from destruction, and this no doubt explains in part at least its efficacy in promoting the absorption of insulin.

Discussion. The results of control experiments indicate that sodium amytal is a suitable anesthetic for the type of work reported here, and indicate how readily correction may be made, in consideration of the effect of the anesthetic, in interpreting the results. The blood sugar of the dog shows a gradual falling off with time after the administration of the anesthetic, and this change can be easily taken into account. The response of the animal to known amounts of insulin shows that, providing the blood sugar is above 50 mgm. per cent, 5 units injected intravenously will bring about a drop of about 20 mgm. per cent in 30 minutes. Using 50 units subcutaneously in an animal of comparable size, an effect but little greater is obtained in this interval of time. Obviously it would be unfair to expect as great a change from alimentary administration as from intravenous. But many experiments show quite as great an effect from the intestinal loop washed free of enzymes and treated with absorptive agents as from subcutaneous injection of the same dose, within the same time.

It is conclusive now that the absorption of insulin is not related to surface tension and hydrotropism, but involves problems which have been inadequately investigated. For example, the calcium ion has been reported to inhibit absorption, but the studies from which this inference was drawn were limited to simple substances like salts and sugars. In this work calgon had a slight effect, but citrate did not, and it is likely that removal of the calcium ion has no effect on the absorption of insulin.

The irritation of a membrane or an increased blood flow will not in themselves bring about an increased absorption of insulin. The effect on the mucosa must be specific because quinine, a "protoplasmic poison," and methyl salicylate were active in isolated loops whereas other irritants were not.

Perhaps of more interest have been consistent reports from this laboratory that a high pH favors absorption. Young, Phillips and Murlin (17) found that the optimum pH range for maximum effect in stomach tube experiments was from 9.9 to 10.5. The data in table 4 show that a high pH is favorable for absorption, and since these data were obtained from

stomach tube experiments, it may be argued that the increased absorption of insulin was due to a protection of the insulin from pepsin by alkali. But Sealock, Murlin and Driver (3) using hexyl resorcinol at a pH of 10 in intestinal loops found an average blood glucose change of 10.3 mgm. per cent compared to a drop of 6 reported in this paper where the pH of the solution was 4.5. Since no pepsin was present in these loop experiments, it seems that the alkali brings about some kind of a change in the mucosa, perhaps nothing more than the familiar softening effect on tissues generally.

Since the efficacy of hexyl resorcinol cannot be explained on a surface tension basis, it is necessary to look for other factors. A comparison (3) of the alkyl resorcinols in their effects on absorption and bactericidal action showed a perfect parallelism between the two, hexyl resorcinol being most effective in both cases. What can be inferred from this parallelism? Höber, Andersh, Höber and Nebel (19) found that hexyl resorcinol depolarized membranes of muscle and nerve. Osterhout (20) found the same thing with Nitella and a simultaneous decrease in the apparent mobility of sodium and potassium. He stated that hexyl resorcinol probably produces structural changes in Nitella, but that the nature of these alterations required further investigation. Höber et al. described the effect of hexyl resorcinol on membrane potential as due to a dispersing effect on the colloids of the surface. The investigations reported here also indicate a change in the membrane. Preliminary results indicate that a combination of hexyl resorcinol with alkali completely reverses a hindering effect of hexyl resorcinol alone on dead or surviving membranes. This combination is always more effective in promoting absorption than the resorcinol in acid medium. Hence, it may be inferred that a still greater "dispersing effect" is caused by the combination, or dispersal plus softening (see above). Probably the combination would have also a greater bactericidal effect.

Pinacol has no appreciable effect either on surface tension or enzyme inhibition, and yet it is one of the most effective agents known in promoting the absorption of insulin. This property is probably due to a favorable combination of hydrophobic and hydrophilic groups in the molecule which enable it to fit into the insulin molecule by a sort of lock-and-key mechanism. It was thought that the hydrophobic groups attached themselves to the insulin molecule leaving the two polar hydroxyl groups exposed exteriorly, thereby making the insulin more soluble and allowing it to penetrate the aqueous part of the membrane more rapidly as has been postulated for the action of bile salts on fat absorption. In order to test this premise, the influence of pinacol on the diffusion of insulin through a sintered glass membrane was studied, but there was no increase in diffusion as there should have been if the hypothesis were correct. Now since at least 30 hydrotropic substances have been found which are without effect,

the question arises as to whether the absorption of insulin is a matter of water solubility alone. Future experiments will explore the possibility of effects from agents which modify lipoid solubility.

SUMMARY

The blood sugar of the dog under amytal anesthesia falls gradually, amounting on the average to about 15 mgm. per cent in 12 hours.

In many experiments with intestinal loops effects on blood sugar quite as great as from subcutaneous administration have been obtained in 30 minutes from the same amount of insulin introduced into the loop with various promoting agents. However, the effect is shorter-lasting, which means obviously that less insulin reaches the circulation from the alimentary tract. Pinacol, Aerosol O.T., methyl salicylate, thiamin and quinine, as well as the alkyl resorcinols, promote the absorption of insulin in this manner at a pH of 4.5. The effect of methyl salicylate is attributed to its special irritating influence on the mucosa. The pinacol molecule probably has a configuration such that it fits into the insulin molecule, thereby bringing about a change in the penetrability of the latter. Alkaline reactions favor absorption still more. Quinine "poisons" the mucosa, whatever this may mean, causing a more rapid penetration of insulin.

The opinion has been reached that there is no direct relationship between the absorption of insulin and surface tension.

A high pH is advantageous in inhibiting the destructive action of pepsin on insulin and in softening the mucosa for its absorption.

Acidic and basic organic dyes and other colloids supposed to precipitate and inactivate enzymes do not promote the absorption of insulin when added to solutions containing hexyl resorcinol.

No effect on absorption is observed by increasing villous activity, adding potassium or removing calcium.

It is possible to obtain an additive effect on absorption by combining different factors.

Hexyl resorcinol promotes the absorption of insulin principally by changing the nature of the membrane and by protecting the hormone from enzymatic hydrolysis.

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A COMPARATIVE STUDY OF EXCISED CEREBRAL TISSUES OF ADULT AND INFANT RATS[1]

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Previous studies (1) from this laboratory have disclosed that the excised cerebral tissue of infant rats 1 to 25 days old possesses a lower oxygen consumption than adult tissue. In the present investigation these results have been extended to include a comparative study of various parts of the adult rat brain, as well as of the effects of alcohol and pentobarbital on adult and infant cerebral tissue.

METHOD. The oxygen consumption of minced cerebral tissues was measured in the Warburg respirometer. The tissue was suspended in saline buffered with phosphate at pH 7.4 and glucose as substrate. The brain of the adult rat was separated into four parts: cerebral cortex, cerebellum, medulla, and the remainder including basal ganglia, thalamus, hypothalamus, and midbrain was termed brain stem. These parts were minced and white and gray matter were thus studied together. The entire brain of the newborn rat (less than 24 hrs. post natal) was used. The various parts of the adult and the entire infant brain were exposed in different experiments to 6 per cent alcohol and 0.012 per cent pentobarbital.

RESULTS. The data presented in tables 1 and 2 reveal that among the various parts of the adult rat brain, the cerebral cortex possesses the highest oxygen uptake (column 3). Brain stem, cerebellum, and medulla follow in the order given. The differences in the metabolic rate are significant.

The effect of 6 per cent alcohol (table 1) is to depress the metabolism of every part of the brain. The cerebral cortex suffers the greatest absolute depression while that of the brain stem is the least (column 5 = column 3 − column 4). On a percentage basis, cortex, cerebellum, and medulla are inhibited approximately to the same extent and brain stem to a lesser degree (column 6).

Pentobarbital in 0.012 per cent solution (table 2) exerts the most profound absolute depression on the cerebral cortex (column 5 = column 3 −

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column 4). The percentage inhibition of the brain stem is less than that of the other parts of the brain (column 6).

TABLE 1

The effect of alcohol on cerebral metabolism of various parts of the adult rat brain

mm.^{3} O_2/100 mgm. tissue/hour

1	2	3	4	5	6
PART	NO. OF OBSERVATIONS	CONTROL	ALCOHOL	DEPRESSION	
				Absolute	Per cent
Cortex	19	316	195	121	38
Brain stem	20	251	204	47	19
Cerebellum	20	179	111	68	38
Medulla	20	161	103	58	36
Total	79				

TABLE 2

The effect of pentobarbital on various parts of adult rat brain

mm.^{3} O_2/100 mgm. tissue/hour

1	2	3	4	5	6
PART	NO. OF OBSERVATIONS	CONTROL	PENTOBARBITAL	DEPRESSION	
				Absolute	Per cent
Cortex	40	278	170	108	38
Brain stem	39	226	163	63	28
Cerebellum	40	167	106	61	37
Medulla	41	147	90	57	39
Total	160				

TABLE 3

The effect of alcohol and pentobarbital on the infant rat brain

mm.^{3} O_2/100 mgm. tissue/hour

1	2	3	4	5	6
NO. OF OBSERVATIONS	CONTROL	ALCOHOL	PENTOBARBITAL	DEPRESSION	
				Absolute	Per cent
16	102	81		21	21
44	105		83	22	22

The oxygen consumption of the brain of rats less than 24 hours old is approximately two-thirds that of the adult medulla (table 3, column 2). Both on a percentage basis as well as absolutely the depressive effects of alcohol and pentobarbital are less in the newly born rats (columns 5 and 6).

Discussion. These results on the rates of metabolism of the various parts of the adult rat brain may be compared with those of Dixon and Meyer (2) on ox brain. The English workers observed the highest rate in the cerebellar cortex and then, in decreasing magnitude, the caudate nucleus, cerebral cortex, thalamus, hypothalamus, and Globus Pallidus. In Dixon and Meyer's experiments the gray matter only was studied and the difference observed in their experiments represented the rates of respiration of gray matter in the various parts of the brain. In the present experiment entire parts consisting of white and gray matter were studied. White matter possesses a much lower rate of metabolism than does the gray. Certainly the medulla with its low oxygen uptake possesses relatively the highest proportion of white matter. It is interesting that despite the difference in experimental materials, with the exception of the cerebellar cortex, the result of Dixon and Meyer in the main agrees with the present observation for the oxygen utilization of the cerebral cortex is greater than the average for the various parts of the brain stem.

An analysis of the oxygen consumption of the various parts of the brain suggests that in general there is an increase of metabolic rate as the neuraxis is ascended. The layers of newer phylogenetic origin appear to possess a greater metabolism. The lower metabolism of the immature brain of the newly born rat is in accordance with this conception. Similar observations have been made on newly born dogs (3). Many differences between infant and adult brain may be partly explained by this lower cerebral metabolism; for example, the greater resistance to cerebral anemia of the infant is probably associated with this smaller oxygen requirement (4). It has also been found that the cerebral electrical activity stimulated in the kitten by metrazol persists during a longer period of cerebral anemia than the activity of the adult cat (5). Perhaps the larger dose of metrazol required to stimulate convulsions in the kitten may also be associated with a lower cerebral metabolism.

Since the metabolic rate of each part of the adult rat brain appears to be characteristic, it was possible to make a comparative study of the effects of narcotics on the various cerebral fractions. There has been much discussion as to the site of action of narcotics, some of them have been classified as exerting their effects chiefly on the cerebral cortex and others on the brain stem (6). Alcohol is regarded by this worker as a cerebral narcotic and the present results reveal that the cerebral cortex suffers the greatest depression by alcohol. However, it should be pointed out that the concentration of alcohol used in these experiments is greater than the toxic dose. This indicates that *in vivo* alcohol produces its effects in other ways in addition to the depression of respiratory metabolism of cerebral tissues.

The effects of pentobarbital occur in much smaller concentration and,

therefore, lend support to Quastel's conception that the pharmacological actions of the barbiturates depend upon their depression of brain metabolism (7). The profound absolute diminution of cortical respiration with pentobarbital indicates that the barbiturates may exert effects chiefly on that portion of the brain and not on the brain stem where the depression is least when reckoned on a percentage basis. Keeser and Keeser (8) reported the greatest concentration of barbiturate in the brain stem. This and other evidence led Pick to conclude that the barbiturates act chiefly on the brain stem. Koppanyi, Dille and Krop (9), however, found similar distributions of barbiturates throughout the various portions of the central nervous system. If the barbiturates are thus equally distributed, then the part of the brain with the greatest oxygen requirement, namely, the cortex, would be the first to succumb to the effects of this narcotic. By the same reasoning the brain of the newly born rat with its lower metabolism might be expected to be more resistant to barbiturates than the adult brain. It may be pointed out that recordings of the electrical potentials of the brain in dogs receiving pentobarbital reveal that the cortex is depressed long before the hypothalamus (10).

SUMMARY AND CONCLUSIONS

1. The various parts of the adult rat brain have different metabolic rates and in the following order, cerebral cortex, brain stem, cerebellum, and medulla.

2. With huge doses of alcohol and pharmacological amounts of pentobarbital the absolute depression of metabolism is greatest in the cerebral cortex. On a percentage basis the brain stem is least affected.

3. Cerebral tissue of newly born rats is less sensitive than that of adults to alcohol and pentobarbital.

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ORIGIN AND EXTENT OF GASTRO-INTESTINAL MOTILITY IN THE CAT AND GUINEA PIG

Direct Observations on Fetuses

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It has been determined by roentgenographic technique that swallowing and defecation in amnio are normal physiologic functions in the guinea pig fetus. Swallowing of amniotic fluid and passage of material along the gastro-intestinal tract have been demonstrated in a few human fetuses. These studies have shed little light upon the nature of early propulsive movements, and the possibility of the occurrence of such motility prior to the time swallowing begins has not been considered (2, 6).

Motility of the excised fetal stomach has been described and active hunger contractions have been recorded in prematurely delivered animals. A few direct observational studies of fetal intestines in situ have been made (6, 10). The latter were based on asphyxiated fetuses and do not represent fairly the status of gastro-intestinal activity in fetuses with placental circulation functioning.

The roentgenologic studies have shown that the gastro-intestinal tract is normally active during the last third of fetal life. The present study will indicate the types of gastro-intestinal activity elicitable at various stages of gestation under the conditions of laboratory experimentation.

Method. Sixty cat fetuses ranging in age from 25 to 64 days (term, 65–67 days) and 51 guinea pig fetuses, 15 to 57 days old (term, 67–69 days) were used.[2] Gestation was dated from verified matings in the laboratory. Twelve of the 18 pregnant animals used were decerebrated by the anemia method (4) prior to delivering their fetuses into a constant temperature bath (37–38°C.) of Locke's solution. This made it possible to proceed without an anesthetic. Placental circulation was maintained. The remaining 6 cats (24 fetuses), threatening abortion, were anesthetized with nembutal, ether, or light ether and procain. The fetuses of aborting cats were examined at room temperature on the lab-

[1] Aided by a grant from The John and Mary R. Markle Foundation.

[2] About 30 additional cat fetuses were studied. The results comprise a preliminary report (8).

oratory table. In two such animals, the placentas were disengaged from the uteri before the fetal intestines were exposed.

The majority (44) of the guinea pig fetuses were delivered into a bath of Locke's solution (usually about 35°C.) subsequent to infiltration of the maternal abdominal walls with 1 per cent procaine. The rest were delivered after nembutal or ether anesthesia. Placental circulation was maintained in all but 2 of the 25 pregnant animals.

In the bath, the delivered fetus was floated upon a submerged platform, its limbs fastened securely by bull-dog clamps or threads and the stomach and intestine exposed through abdominal incisions. The slender intestines of the younger specimens were observed through a binocular microscope magnifying 36 times. For older fetuses, a large reading glass proved helpful. In some experiments the umbilical cord was temporarily or permanently occluded to observe the effects of asphyxia upon gastrointestinal motility. Motion picture records of typical experiments were made.

It was important to keep the fetal organs submerged. Protrusion from the bath often led to severe, localized, intestinal knotting. The temperature of the bath could be lowered several degrees without affecting intestinal motility, but one or two degrees' elevation often led to diminished tone and quiescence.

Results. Cat fetuses ranged in size from 16.5 mm. to 130 mm. crown-rump length (0.5 to 91.0 grams). Until about the 35th day of gestation, the amniotic fluid was usually clear and abundant; in two instances it was straw-colored. Intestines were white and no sharp differentiation between large and small intestine was possible at this early stage. Between 35 and 40 days, the amniotic fluid varied from straw color to a deep yellow. Toward the end of this period swallowing may have begun for the small intestine likewise appeared yellow in color while the large intestine remained white. The small intestine first appeared definitely thicker than the large intestine on the 45th day, and at this time it contained an abundant soft, yellow material; the large intestine was clear and apparently empty. By the 55th day the amniotic fluid had a greenish tinge, and there was less of it than there had been earlier. The small intestine was packed with meconium. Meconium usually filled the proximal part of the large intestine although several fetuses still showed an empty colon and yellow fluid occupied the amniotic sac. In 60 to 64 day fetuses, only a cubic centimeter or less of free fluid was found. Green meconium plastered the body of the fetus. The large bowel was packed with semi-fluid meconium.

Under optimum experimental conditions, no spontaneous gastrointestinal activity was found in 25 day old cat fetuses (16.5 mm.), although the intestines contracted locally when pinched lightly. True intestinal

peristalsis was present in 28 day (23 mm.) fetuses, though it was more frequently encountered in 29 and 30 day specimens (27 to 28 mm.). Gastric peristalsis was present for the first time on the 29th day. The constrictions were deep, and moved from the middle of the stomach toward the pylorus. In the 35 to 40 day old (36 to 65 mm.) fetuses a contraction wave passed over the stomach every 15 seconds; gastric activity persisted as long as three-quarters of an hour in the water bath. Rhythmic segmentation predominated in the small intestine, although intervals of moderate peristalsis were common. Gastric peristalsis was rarely encountered and motility in the small intestine was sluggish in fetuses older than 50 to 55 days (90–110 mm.). This condition persisted until birth. The large intestine was singularly inactive at all ages as long as observations were conducted in the water bath. Nevertheless, meconium was found in the amniotic sac at least as early as the 58th day (120 mm.).

Clamping the umbilical cord led to a change in type of gastrointestinal activity even in cat fetuses as young as 35 days (36 mm.). Gastric peristalsis ceased within two minutes. If intestinal peristalsis preceded clamping, it gradually gave way to a generalized rhythmic segmentation involving the entire intestine. Usually, within 15 minutes after complete occlusion of the umbilical vessels, segmentation diminished, the gut smoothed, and writhing pendulous movements appeared; these persisted even after death of the fetus. Some festuses showed no spontaneous activity in the intestine until the cord was clamped. Then rhythmic segmentation commenced, lasted only momentarily, and within ten minutes the intestine again became quiet, although severely constricted. A normally inactive intestine could be excited to activity temporarily by lightly clamping the cord. Restoration of placental circulation led to normal quiescence within two or three minutes. Similarly, intestinal peristalsis could be abolished in favor of generalized rhythmic segmentation, only to reappear after the clamp was released. However, when the intestines were active, permanent obliteration of the umbilical circulation always finally led to pendulous, agonal writhing.

Deep ether anesthesia administered to the pregnant mother before delivery rendered the fetal gastro-intestinal tract entirely unresponsive even to mechanical stimulation. When the fetuses were quickly removed after light ether anesthesia and local procaine, the intestines responded with immediate hyperactivity just as they did when the umbilical cord was first clamped. Within 15 minutes, a diminution of intestinal tonus ensued. Nembutal anesthesia, on the other hand, led to a general depression of all fetal activity, lasting as long as 30 minutes in some cases. A period of hyperactivity followed; gastric peristalsis, rhythmic segmentation and occasional intestinal peristalsis seemed brisker than that normally encountered. These effects were noted in specimens as young as 33 days'

gestation (35 mm.). When a general anesthetic had been administered, anastalsis and nondirectional propagation were encountered part of the time in the fetuses younger than 45 days old (75 mm.). Gastric peristalsis often suddenly reversed and proceeded toward the cardia. In the intestine, peristaltic waves traveled both orally and aborally from a constriction. Sometimes the direction of propagation suddenly reversed in several loops of intestine while it continued aborally in others.

Guinea pig fetuses ranged from 5 mm. to 120 mm. (0.15 to 87 grams) crown-rump length. The amniotic fluid was colorless until about the 42nd day of gestation. By the 36th day, the cecum clearly marked the division between small and large intestines although it had not yet become sacculated. By the 50th day, the small intestine was light yellow in color and the amniotic fluid was a darker yellow. The cecum and proximal colon were white and sacculated as far as the descending colon. The latter was colored a deep yellow to brown and had a knotted appearance although its contents were soft. Between 55 and 60 days, the amniotic fluid contained particles of light yellow meconium. Between 60 and 67 days, the fluid was sparse and contained a deep yellow or green colored meconium. The small intestine was usually empty and inactive during this period of development, but the large intestine was distended and, at 60 days, was packed with a soft yellow mass. Near term, meconium of a more solid nature than heretofore appeared in knot-like constrictions along the descending colon.

No gastro-intestinal motility was observed in the fetal guinea pig until the 27th day (17 mm.). At this time local contractions appeared when the intestine was pinched lightly. Rhythmic segmentations of short duration were noted on the 29th day (22 mm.) during asphyxia (placentas of these fetuses had been separated from the uterus). True gastric and intestinal peristalsis was not encountered until the 35th day (35 mm.) and was sluggish. By 40 days (48 mm.) peristalsis was seen in both the large and small intestine, more frequently in the latter. The cecum contracted rhythmically in 42 day (50 mm.) specimens. Gastric peristalsis was still sluggish and arrhythmic. It became more coördinated and rapid by the 45th day (67 mm.). At 50 to 55 days (80 to 95 mm.) peristalsis was found mainly in the ascending colon. When it occurred with localized rhythmic segmentation of the small intestine, it carried the intestinal bolus for a distance of 8 to 10 mm. In the proximal colon, the bolus was sometimes moved as much as 2 cm. so rapidly that it simulated a peristaltic rush. Usually, however, the proximal colon appeared tonically constricted and blanched but was otherwise inactive. Gastric peristalsis, when present, was about as active as in the newborn. Contraction waves passed from the middle of the corpus over the pyloric antrum at the rate

of 1 every 3 seconds. Most activity in 60 to 67 day (110–120 mm.) fetuses was restricted to the large intestine. Peristaltic rushes were frequent, carrying the contents from cecum to descending colon. Segmentation was prominent in the proximal colon. Activity was rarely seen in the small intestine. Gastric peristalsis, when observed, was always brisk.

The gastro-intestinal tract of the fetal guinea pig responded to experimentally induced asphyxia and to general anesthesia exactly as it did in the fetal cat.

COMMENT. The fact that the intestinal musculature of both the cat and guinea pig fetus responded to light pinching before spontaneous activity was present agrees with Yanase's observations (10) on freshly killed guinea pig fetuses 15 mm. long. He reported that peristalsis appeared in the 19 mm. guinea pig, but it is not clear that he meant propagational movements. Under the best of our experimental conditions in the water bath with placental circulation intact we were unable to observe true peristalsis in the guinea pig until the 35th day (35 mm.). Rhythmic segmentation did appear in younger specimens but only under poor conditions (impaired placental circulation).

Apparently gastro-intestinal activity begins later and at a lower level of activity in the fetal guinea pig than in the cat. From the 50th day to term, however, gastro-intestinal motility functioned at a more mature level than in the fetal cat of comparable age. Species differences in motility may have been due to structural differences in the cecum and colon. In the cat during the last quarter of gestation the simple, tube-like cecum and colon were distended with meconium, peristalsis was infrequent, and rhythmic segmentation seemed restricted to the small intestine. Apparently defecation in amnio is not as common in the cat as in the guinea pig (2). Perhaps distention of the lower bowel acted reflexly in the cat fetus to inhibit motility in the stomach and proximal intestine. In the guinea pig, on the other hand, the cecum and proximal colon were large, sacculated, and active. In specimens 55 days or older the small intestine was often empty, and mass peristalsis was commonly seen in the large bowel. That defecation and reswallowing of fluid may occur as often as five times before birth has been reported (2).

Motility of the fetal gastro-intestinal tract seems to follow a definite developmental pattern. The earliest activity encountered was strictly localized. It consisted of contraction and relaxation following mechanical or electrical stimulation of the gut. Except for its slowness of execution, it was much like the earliest localized somatic movements of the mammalian embryo (5, 7, 9). Whether a local rhythmic segmentation or a true peristalsis is the next step in development was impossible to determine with certainty. Under the best of conditions in 9 of the 15 early cat

embryos (28–30 days old) peristalsis was observed in intestines which showed no localized rhythmic segmentation. In the remaining 6 embryos studied in air or with placentas detached, segmentation predominated but there were sporadic periods of peristaltic activity. These changes were attributable partly to asphyxia. In 3 of 5 early guinea pig fetuses (35–36 days old) peristalsis was present as soon as the intestines were exposed, but within twenty minutes rhythmic segmentation also appeared. Even before this, rhythmic segmentation alone was elicited by asphyxia in three fetuses (29 days). Often it was possible in older specimens to observe both phenomena in the same intestine. However, neither rhythmic segmentation nor peristalsis, complex patterns of behavior, appeared to be established until several days after the gut was able to respond locally.

TABLE 1

Summary of events in the development of G.-I. motility

	CAT		GUINEA PIG	
	Days	Millimeters	Days	Millimeters
1. Marked vagal innervation of stomach and sympathetico-vagal innervation of intestines	23	13		
2. Enteric plexuses and smooth muscle present. Local contraction of intestine to pinching	25	16.5	27	17
3. Rhythmic intestinal segmentation induced by anoxemia	28	23	29	23
4. True intestinal peristalsis began	28	23	35	35
5. True gastric peristalsis began	29	27	35	35
6. Colonic peristalsis began	42	70	40	48
7. Defecation began	58	120	55–60	95–110

In a preliminary report, Windle and Bishop (8) offered an explanation for the changes in gastro-intestinal behavior which ensue during anoxemia. They correlated changes in the oxygen content of umbilical vein blood with changes in motility. The present results are in accord with these. Similar changes in fetal somatic motor responses during anoxemia have been described (7). Other examples of smooth muscle activation during partial anoxemia (at birth) may be the constriction of the ductus arteriosus sphincter and the contraction of the fetal spleen (6).

Burstein (3) has recently examined the effects of the barbiturates, including nembutal, upon intestinal activity in adult dogs with Thiry-Vella loops of the upper jejunum. Balloon tracings recorded an immediate depression of rhythmic intestinal contraction and a fall in tonus lasting

from 5 to 15 minutes. The primary effect was transient and was succeeded by a more prolonged phase of heightened intestinal contraction and tonus. Evidently, the fetal intestinal musculature responded like the adult in this respect.

Alvarez (1) has reported that stimulation of the small intestine of the rabbit at any point gives rise to a wave of contraction which travels both orally and aborally. A similar condition was encountered in several of the fetal cats and guinea pigs early in gestation; this reaction was spontaneous. Whether such behavior is really a refutation of the "law of the intestine" or not is difficult to say; in our experiments it occurred only under duress of general anesthesia.

Although histological study of the gastro-intestinal tracts of the fetuses is still incomplete, a few interesting facts have been ascertained. By the time the fetal gastro-intestinal tract of the cat and guinea pig begins to exhibit peristalsis, both circular and longitudinal muscle are present in the stomach and intestine. The myenteric and submucous ganglia and plexuses are present for several days before gastro-intestinal motility begins. In fact they are present before local constrictions are first elicitable. In 13 mm. to 18 mm. cat embryos, a rich vagal supply can be traced to the stomach and a prominent sympathetico-vagal supply leaves the coeliac ganglia for distribution to the intestinal tract.

SUMMARY

1. The earliest indication of motility in the gastro-intestinal tract of both the fetal cat and guinea pig was a simple localized contraction in response to stimulation (25–27 days, approximately 17 mm.).

2. Spontaneous gastric and intestinal peristalsis was present in cat fetuses of 29 to 30 days' gestation (27–28 mm.) and in guinea pig fetuses on the 35th day (35 mm.).

3. Peristalsis was observed earlier than rhythmic segmentation in the cat, but both types of motility often were present in the same intestine. Segmentation was elicitable before spontaneous peristalsis appeared in the guinea pig.

4. Peristalsis became progressively more active in the stomach and small intestine up to about 50 days' gestation. Thereafter it became less active in the small intestine of both species. In the guinea pig, however, the stomach, cecum and colon retained a high degree of motility throughout.

5. After clamping the umbilical cord, peristalsis gave way to generalized rhythmic segmentation and then to diminished intestinal tonus with slow, pendulous writhing of the intestine. Anoxemia likewise excited inactive intestines to an initial hyperactive state of generalized rhythmic contrac-

tion and relaxation; soon activity ceased, but the intestines remained severely contracted for some time.

6. Light ether anesthesia had an effect upon the fetal intestine like that engendered by clamping the cord. Deep ether anesthesia inhibited activity completely.

7. Nembutal administered to the pregnant mother first depressed fetal gastro-intestinal activity; gradually tonus and rhythmic motility returned and finally the gut became hyperactive.

8. Anastalsis and general nondirectional activity were characteristics of the hyperactive state during anesthesia in young fetuses of both species.

9. By the time peristalsis was established in the fetal cat and guinea pig, circular and longitudinal muscle surrounded the gastro-intestinal tract.

10. The myenteric and submucous ganglia and plexuses were established as early as 17 mm. in both species, while a rich vagal supply to the stomach and an equally prominent sympathetico-vagal supply from the coeliac ganglion to the intestinal tract were present in the cat at 13 mm.

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CERTAIN QUANTITATIVE ASPECTS OF THE PANCREATIC RESPONSE TO SECRETIN

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In the very large number of secretin assays performed in this laboratory according to the method described by us (1), we have repeatedly observed that there was no direct proportionality between the amount of secretin injected and of the resultant output of pancreatic juice. Thus, it has been noted that doubling a small dose of secretin will approximately treble the secretory response. It has also been our impression that the reaction to injections of isolated quantities of secretin does not resemble the condition existing when the pancreas secretes in response to a meal. There is no information at present regarding the minimum quantity of secretin which must be present in the circulation in order *a*, to cause the pancreas to secrete at a minimal and constant rate, and *b*, to cause the gland to secrete at its maximal rate.

The following experiments have been performed with a view to clarifying these problems.

Experimental. Intact dogs were anesthetized with sodium pentobarbital, the femoral vein exposed, and the main pancreatic duct cannulated in the usual manner. The secretin used was our SI preparation, 0.25 mgm. of which stimulates the pancreas of over 50 per cent of dogs to secrete 10 drops when injected intravenously, or represents a dog unit (1) or "threshold dose." The volume response of the pancreas to graded doses of the secretin was measured by collecting the pancreatic flow in a small graduated cylinder and noting the volume obtained during the first 10 minutes after injection and also for the entire duration of action. The animals were always permitted to return to the basal level after each stimulation of the pancreas. Injections were made at a rapid rate.

A second series of dogs was prepared in a similar manner, except that secretin solutions of graded concentration were injected into the femoral vein at a constant rate by a Woodyatt pump. The secretory response was measured in drops.

Results. *Threshold dose of secretin.* The usual individual variations were noted in the 25 dogs used in these experiments. The threshold dose

was 0.2 mgm. in two dogs, 0.25 in twelve, 0.5 in six, 1.0 in four, and 1.5 in one. The percentage distribution of the animals is shown in figure 1.

Effect of single secretin doses in increasing amounts. In the 25 animals studied there was uniformly a sharp increase in the quantity of flow in response to increasing secretin dosage. With further secretin additions the secretory increase became progressively less marked and finally a

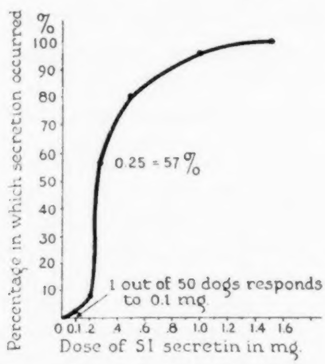

Fig. 1. Distribution curve in 25 dogs showing individual variations in threshold dose of secretin.

TABLE 1

INJECTION (THRESHOLD DOSES)	SECRETORY RESPONSE		Δ		DURATION OF ACTION
	1st 10 min.	Total	1st 10 min.	Total	
	cc.	*cc.*	*cc.*	*cc.*	*minutes*
1	0.4	0.4	0.4	0.4	10
2	1.2	1.4	0.8	1.0	20
3	1.9	2.3	0.7	0.9	25
4	2.5	2.9	0.6	0.6	29
5	3.1	3.4	0.6	0.5	32
6	3.4	3.9	0.3	0.5	35
8	3.7	4.5	0.15	0.3	34
10	4.2	5.2	0.25	0.35	33
12	4.3	5.6	0.05	0.2	33
14	4.4	6.0	0.05	0.2	35
16	4.6	5.9	0.1	−0.05	33

secretin dosage was obtained which stimulated the pancreas to its maximum capacity. Beyond this point further increases in secretin administered elicited a fairly constant response. The average responses in the 25 animals are listed in table 1. In this table are summarized the secretory responses for the first 10 minutes, the total response, the duration of action of the secretin, and the value designated by us as Δ, or the increase in secretion per unit increase in secretin threshold doses.

Effect of continuous injection of secretin in increasing amounts. This technique was carried out on 5 dogs. The results are tabulated in table 2.

DISCUSSION. The individual variations in threshold dosage of our SI preparation were of the same order as those previously observed by us in very many animals. Since this information has never been published and since it was available on the 25 dogs studied in this work, we are including it in the present report.

It is evident from the findings tabulated above that no simple proportionality exists between the magnitude of a given dose of secretin and the pancreatic response. In all cases the effect of increasing single doses of secretin was an initial sharp increase in pancreatic secretion, followed by a gradually lessening increase in response until a maximum flow was obtained which could not be exceeded by any further increase in administered secretin. The average responses for the 25 dogs tested are represented in figure 2. The relationship between dosage and degree of response is a

TABLE 2

SECRETIN THRESHOLD DOSES PER MINUTE	RESPONSE IN CC. PER 15 MINUTES				
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
0.05					0.3
0.10	1.5	1.0	0.8	1.0	0.6
0.20	5.6	2.4	1.0	1.5	2.7
0.30				2.5	5.7
0.40	5.7	9.0	2.3	3.1	9.0
0.60		12.8	5.0	4.1	13.0
0.80		11.2	6.2	5.0	15.2
1.00			7.5	4.3	15.9

curvilinear one in which the curve assumes an S-shape. The same type of curve is obtained both for the 10-minute and the total responses; the former reached its maximum value at a lower dosage level than did the latter. It was also noted that although, as expected, the action was more prolonged in higher than in lower dosages, the duration of action reached a maximum before the total flow did so.

The S-shaped curve (fig. 1) denoting the variation in the threshold dose in different animals is to be expected (2). The concentration-action curve (fig. 2) is also S-shaped. This might also be expected since when a minimal effective amount of secretin is injected the response is small, and when the dosage of secretin is progressively increased above this minimal one the pancreatic output is elevated to a degree out of proportion to the increase in dosage. Thus when a curve is plotted with secretin dosage as the abscissa and pancreatic response as the ordinate there is at first a gradual, then a sharper rise in response; and finally the rise again becomes gradual

and levels off when a secretin dosage is attained which stimulates the gland to secrete at its maximum capacity.

These results demonstrate that it is fallacious to assay unknown secretin preparations by administering sizable amounts to an animal and interpreting the dosage by calculation on the basis of direct proportionality in terms of a very small and arbitrarily fixed response (3).

The experiments in which graded doses of secretin were injected continuously at uniform rates yielded results consistent with those obtained by single injections in that as the quantity of secretin injected per unit of time was increased there was at first a rapid, then a more gradual increase in volume output of secretion. The minimum effective dose found was 0.05 threshold dose per minute and a maximal flow was obtained at 1 threshold dose per minute. Since in previous experiments it has been noted by us (1) that the threshold dose of pure secretin base in the dog is

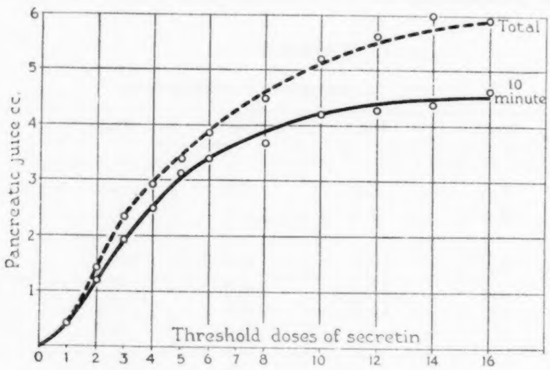

Fig. 2. Total and 10-minute volume output of pancreatic juice in response to increasing doses of secretin.

0.014 mgm., it follows that the minimum amount necessary to be present in the circulation of an *anesthetized* dog is 0.7 gamma, and the amount required to cause the gland to secrete at its maximum rate is 14 gamma. These statements are based on the assumption that when secretin is injected at a constant rate a condition of equilibrium is attained in the animal so that the rate of "destruction" equals the rate of injection and a constant amount of secretin is present in the circulation for any given continuous dosage less than that quantity which exerts a maximal effect. The constant secretory rates which we have found appear to bear out this assumption, the doses of secretin used being less than supramaximal.

SUMMARY AND CONCLUSIONS

The volume response of the pancreas to graded doses of secretin has been studied. When secretin is given in single doses in increasing amounts

the pancreatic flow increases; this increase takes place rapidly when the dosage is small and gradually when it is large. A dosage is finally obtained which causes the gland to secrete at its maximum capacity and beyond which further increases in the quantity of secretin injected give no greater response. The increase in volume output when measured for the first 10 minutes was similar to that noted for the total duration of action. Any assay method based on the assumption that a direct proportionality exists between the amount of secretin injected and the amount of juice secreted is fallacious, since the relation is curvilinear. When secretin was given continuously in increasing amounts, the results were analogous to those obtained with single doses. It is concluded from the continuous injection experiments that the minimal effective amount of pure secretin base necessary in the circulation of the average anesthetized dog is 0.0007 mg. and the amount requisite to stimulate the gland to secrete at its maximum capacity is 0.014 mgm.

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